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CORRELATION BETWEEN MOVEMENT OF THE CURLY TOP VIRUS AND TRANSLOCATION OF FOOD IN TOBACCO AND SUGAR BEET [1]

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INTRODUCTION

Evidence was presented in a previous publication (*3*) [2] tending to show that the curly top virus occurs in relatively high concentrations in the phloem of affected plants and that it may be absent or present only in low concentrations in other types of tissue. It was shown further that the insect vector, *Eutettix tenellus* (Baker), probably introduces the virus directly into the phloem through its feeding activities. The rate and direction of movement of the virus immediately following introduction into the phloem suggest a relation between virus movement and food translocation. This paper presents results of further experiments designed to throw additional light on this apparent relation.

REVIEW OF LITERATURE

A correlation between virus movement and food translocation has been suggested in the case of a number of virus diseases. It was shown (*1*) that the virus of leaf curl produced symptoms on the new growth of inoculated canes of raspberry plants and passed downward into the root system in the course of a few days or weeks. However, under normal conditions, the virus did not invade the noninoculated canes of the plants during the current season. The following spring, shortly after growth began, the virus entered all of the noninoculated canes of each plant. A movement of the virus into noninoculated canes during a current season could be induced by pruning the canes sufficiently to cause growth of lateral buds. The virus of yellow mosaic of raspberry reacted in a similar way (*2*). Neither virus passed a ringed portion of the stem.

Holmes (*12*) found that the virus of tobacco mosaic, when introduced into mature tobacco leaves, moved toward the stem more rapidly than in the opposite direction, but when introduced into very young leaves it moved more rapidly toward the leaf tip. The path of the virus could be changed by shading leaves or portions of leaves. However, the virus moved out of shaded leaves rapidly. The results obtained indicate that virus movement has some direct or indirect connection with carbohydrate supply, but since the virus was able to move quickly out of leaves starved with respect to carbohydrates it was suggested that the relation may be indirect.

[1] Received for publication July 28, 1936; issued May 1937.

[2] Reference is made by number (italic) to Literature Cited, p. 501.

Samuel (*16*) studied the movement of tobacco mosaic virus out of mature tomato leaves attached to the stem midway between the top and root. He found that the virus almost invariably moved down to the roots before it moved to the top, except when there were fruit clusters above the inoculated leaf. Downward movement was soon followed by invasion of the upper part of the plant. Samuel states (*16, p. 109*) that if virus movement is a true indicator of food movement, this "subsequent movement to the top of the plant would suggest that the metabolites used at the growing point came, not directly from the leaves, but via the roots."

Caldwell (*5*) observed that when tobacco leaves were inoculated with tobacco mosaic virus and kept in the dark the virus passed out of the leaves almost as rapidly as it moved out of normal leaves in the light. He observed further that the virus showed no greater tendency to move into leaves in the dark than into comparable leaves in the light. He concluded that there is no relation between the movement of tobacco mosaic virus and food translocation and that rate of movement is correlated with rate of reproduction of the virus.

When the virus of tobacco mosaic was inoculated into the base of stems of tobacco plants, Grainger (*11*) found that it traveled first to the growing point and to the young leaves, where it multiplied and spread to the older leaves progressively from the top of the plant. The virus seemed to move at a logarithmic rate, and movement did not seem to be correlated with the movement of food or water.

INVASION OF THE SUGAR-BEET PLANT BY THE VIRUS

SEEDLING PLANTS

When the curly top virus is introduced into the distal end of a cotyledon of a seedling sugar beet (*Beta vulgaris* L.) it passes rapidly into other portions of the plant. Beets inoculated by means of a single leafhopper sometimes show translucent veins in the first pair of true leaves in 30 hours. Severin (*17*) showed that in some instances the virus moved from an inoculated cotyledon into an opposite leaf on a seedling plant in 48 hours. This evidence indicates not only that the virus travels rapidly but also that it multiplies rapidly in seedling beets.

To obtain information regarding the completeness and rapidity of virus invasion of the above-ground parts the following experiment was made: Vigorously growing seedling beets with the first pair of true leaves one-fourth to three-fourths of an inch long were used. A viruliferous leafhopper in the nymphal stage and a nonviruliferous adult were caged on the distal half of one cotyledon of each plant, and a nonviruliferous adult was caged on the distal half of the opposite cotyledon. After 24 hours the nonviruliferous leafhopper caged on the cotyledon opposite the one on which the viruliferous nymph fed was placed on a healthy beet. At the same time, the adult leafhopper from the inoculated cotyledon was transferred to a new cage and was placed on a healthy plant, the viruliferous nymph being discarded. This technique was followed for each plant in the test. Other nonviruliferous leafhoppers were caged singly on each of the cotyledons of the inoculated plants for 24 hours, after which they were transferred to healthy plants. Six such lots were transferred on successive days.

The results of this experiment (table 1) show that the leafhoppers were able to pick up virus from the inoculated cotyledon during the

first 24-hour period and that virus was present in the distal half of the opposite, noninoculated cotyledon, in some cases in the second 24-hour period. Apparently the virus invaded most of the parts of the seedling beets very soon after introduction.

TABLE 1.—*Spread of virus from inoculated cotyledon to opposite cotyledon in seedling beets*

[Period of exposure to vector (nonviruliferous leafhopper) 24 hours]

Cotyledon from which leafhopper was taken	Plants infected [1] by leafhoppers transferred from inoculated plants after movement period of—					
	1 day	2 days	3 days	4 days	5 days	6 days
	Number	*Number*	*Number*	*Number*	*Number*	*Number*
Inoculated (check)	10	7	6	10	10	11
Opposite	0	4	8	10	8	9

[1] 20 plants inoculated in each test.

LARGER PLANTS

When large beet plants are infected by viruliferous leafhoppers placed on one of the mature leaves, symptoms of disease almost invariably appear on the new leaves on the side of the crown to which the inoculated leaf is attached. Frequently, some of the new leaves in the earlier stages of attack by the disease show symptoms on only one side, indicating an unequal distribution of the virus in at least the earlier stages of development of the leaf. In some cases only isolated spots showing translucent veins are evident. In later stages of the disease all new leaves show symptoms. The leaves that are mature at the time infection takes place produce no typical symptoms, although they may turn yellow and die earlier than leaves of similar age on normal plants. Whether yellowing and dying are secondary or direct effects produced by the virus has not been definitely determined, but it seems probable that they are secondary effects following injury to other parts of the plant.

No evidence regarding virus multiplication in mature leaves is available, but a certain amount of information has been obtained regarding the relative virus content of mature and immature leaves at different periods following infection. Using beets with 10 to 14 leaves, Severin (*17*) found that the virus moved from an outer inoculated leaf and was picked up by leafhoppers from the inner leaves 10 days later. When the inner leaves were inoculated no virus was recovered from the outer leaves of the plants in 10 days. Carsner and Stahl (*6*), using field beets, caged nonviruliferous leafhoppers on young diseased leaves and on symptomless mature leaves of the same plants and found that in all instances the leafhoppers obtained virus from the leaves showing symptoms. In some instances, virus was recovered from the mature leaves and in others no virus was recovered. Where virus was recovered from the mature leaves the incubation period of disease was longer in plants infected from these leaves than in plants infected from the small leaves showing symptoms.

Experiments were planned to supplement the information available on movement of virus in large beets. Potted plants were allowed to grow until they had a root diameter of 1 to 2 inches. One mature leaf on each plant was inoculated by allowing 50 viruliferous leafhoppers to feed on it 2 days. At the beginning of the period of inoculation a

leaf similar to the inoculated leaf in size and age, but on the opposite side of the plant, was marked.

At weekly intervals for 4 weeks following inoculation, one plant was tested for virus content of the inoculated leaf, the opposite leaf, and the new growth. Four such experiments were performed. In these tests, nonviruliferous leafhoppers were allowed to feed 24 hours on the part to be tested and then caged singly on seedling beets. Thus each experiment used 60 test beets, 20 for each plant part being tested for presence of virus. The infection resulting served to indicate the relative amount of virus present in the different leaves.

The results of the four repetitions of this test are shown in table 2. These results indicate that the virus multiplied in the mature inoculated beet leaves and also that a relatively small amount of virus passed into mature leaves on the side of the plant opposite the inoculated leaf. In some instances the virus apparently did not enter the mature noninoculated leaf in 4 weeks.

TABLE 2.—*Movement of virus in beet plants with roots 1 to 2 inches in diameter*

[Results for each plant part tested in each experiment based on 20 test plants; exposure period to vector, 24 hours]

Experiment no.	Leaves from which leafhoppers were taken	Plants infected by leafhoppers transferred to test plants after movement period—			
		1 week	2 weeks	3 weeks	4 weeks
		Number	*Number*	*Number*	*Number*
1	Inoculated	1	5	9	11
	Opposite	0	0	1	1
	Young	0	7	17	15
2	Inoculated	0	6	13	12
	Opposite	0	0	1	3
	Young	0	12	13	15
3	Inoculated	8	11	16	17
	Opposite	0	3	0	0
	Young	2	16	14	13
4	Inoculated	14	11	12	17
	Opposite	1	2	0	1
	Young	19	17	18	17

RATE OF UPWARD AND DOWNWARD MOVEMENT OF VIRUS IN TOBACCO STEMS

The maximum rate thus far determined for movement of curly top virus downward from the top of Turkish tobacco (*Nicotiana tabacum* L.) plants is one-half inch per hour (24 inches in 48 hours). Further tests were made to compare the downward movement in Turkish tobacco with upward movement.

Tobacco plants approximately 2 feet tall were divided into two lots. The plants of the first lot remained upright and were inoculated at the top. Daily for 7 days following inoculation, five plants were severed at the ground level and defoliated, and the stems were cut into 3-inch segments, which were numbered consecutively, beginning at the top of the plant and planted in sand.

The plants of the second lot were bent so that the stems were approximately horizontal. This treatment induced growth of buds at the base of the stem. All buds except the lowest one on each plant were removed. When the remaining bud had developed into a shoot

approximately 2 inches long, it was inoculated with the curly top virus. At weekly intervals stems of five plants were severed one-half inch above the base of the inoculated shoot. The leaves were removed and the stems were cut into 3-inch segments. The segments were numbered consecutively, beginning at the base of the stem, and then planted in sand.

Growth from the planted segments was rapid, and distinct curly top symptoms were evident on the first leaves from segments carrying the virus. The presence of symptoms on this new growth of the numbered segments served to indicate the distance the virus had traveled during the time interval allowed for movement.

The results of this experiment are shown in table 3. Since the virus moved the full distance downward in every instance in 5-day and longer periods in the plants inoculated at the top, only the results of the 5-day tests are shown. In the series inoculated at the base, movement of the virus away from the point of inoculation required a much longer time than was required in the series inoculated at the top. The time for complete upward invasion varied from less than 14 days to more than 35 days. It is rather striking that with two exceptions the virus either moved the entire distance to the top in the time interval used or did not move a distance of one-half inch from the base of the inoculated sucker. In the two exceptions, the virus was confined to the segment next to the inoculated sucker. This indicates that certain requirements must be satisfied before the virus begins its upward movement and that when the conditions become favorable the upward movement is quite rapid. It should be noted that the plants inoculated at the base had blossomed and set seed before the experiment was terminated. Vickery et al. (*19*) have shown that as the tobacco plant flowers and fruits there is a marked movement of both inorganic and organic materials from the rest of the plant, particularly from the leaves, into the developing capsules. It is suggested that this upward flow of food material toward the top at fruiting time may have been an important factor in the final movement of the virus toward the top of the plants.

To avoid the complicating factor of seed production before termination of the experiment, a second series of tests was conducted in which *Nicotiana glauca* Graham was used as the stem through which the virus passed. Infected segments of stems of *N. tabacum* were used to introduce the virus into the base or top of the plant as desired, and healthy segments of *N. tabacum* were grafted at the end opposite the inoculated end to serve as indicators of the presence of virus.

Potted plants of *Nicotiana glauca* were defoliated and pruned to a height of 3 feet. The plants were divided into two lots, one of 14 and the other of 22 plants. Diseased stems of *N. tabacum* were grafted into the base of seven plants of the first lot, and healthy stems were placed at the top. In seven other plants, the positions of the respective grafts were reversed. These grafts were made immediately after the plants were pruned.

The second lot of plants was kept 2 weeks after pruning before they were grafted to *Nicotiana tabacum*. This allowed time for the production of leafy shoots at the top. Grafts were placed as in the first lot, half of the plants being inoculated at the base and half at the top.

TABLE 3.—*Upward and downward movement of curly top virus in Turkish tobacco*

Plant no.	Period from inoculation to cutting	Direction of movement	Infection (+) or noninfection (−) of—													
					3-inch segment of stem [1]											
			Root	Inoculation part	First	Second	Third	Fourth	Fifth	Sixth	Seventh	Eighth	Ninth	Tenth	Eleventh	Twelfth
	Days															
1	5	Top to root [2]	+	+	+	+	+	+	+	+	+	+	----	----	----	----
2	5	do	+	+	+	+	+	+	+	+	+	+	----	----	----	----
3	5	do	+	+	+	+	+	+	+	+	+	+	----	----	----	----
4	5	do	+	+	+	+	+	+	+	+	+	+	----	----	----	----
5	5	do	+	+	+	+	+	+	+	+	+	+	----	----	----	----
6	7	Root to top [3]	+	+	−	−	−	−	−	−	−	−	−	−	−	----
7	7	do	+	+	−	−	−	−	−	−	−	−	−	−	−	−
8	7	do	+	+	−	−	−	−	−	−	−	−	−	−	−	−
9	7	do	+	+	−	−	−	−	−	−	−	−	−	−	−	−
10	7	do	+	+	−	−	−	−	−	−	−	−	−	−	−	----
11	14	do	+	+	−	−	−	−	−	−	−	−	−	−	−	−
12	14	do	+	+	−	−	−	−	−	−	−	−	−	−	−	−
13	14	do	+	+	−	−	−	−	−	−	−	−	−	−	−	−
14	14	do	+	+	+	+	+	+	+	+	+	+	+	+	+	----
15	14	do	+	+	−	−	−	−	−	−	−	−	−	−	−	----
16	21	do	+	+	+	+	+	+	+	+	+	+	+	+	+	+
17	21	do	+	+	−	−	−	−	−	−	−	−	−	−	−	−
18	21	do	+	+	−	−	−	−	−	−	−	−	−	−	−	----
19	21	do	+	+	+	+	+	+	+	+	+	+	+	+	+	----
20	21	do	+	+	+	+	+	+	+	+	+	+	+	+	+	+
21	28	do	+	+	−	−	−	−	−	−	−	−	−	−	−	−
22	28	do	+	+	−	−	−	−	−	−	−	−	−	−	−	----
23	28	do	+	+	+	+	+	+	+	+	+	+	+	+	+	+
24	28	do	+	+	+	+	+	+	+	+	+	+	+	+	+	----
25	28	do	+	+	+	+	+	+	+	+	+	+	+	+	+	+
26	35	do	+	+	−	−	−	−	−	−	−	−	−	−	−	−
27	35	do	+	+	+	+	+	+	+	+	+	+	+	+	+	----
28	35	do	+	+	+	+	+	+	+	+	+	+	+	+	+	+
29	35	do	+	+	+	+	+	+	+	+	+	+	+	+	+	----
30	35	do	+	+	+	+	+	+	+	+	+	+	+	+	+	----
31	42	do	+	+	+	+	+	+	+	+	+	+	+	+	+	+
32	42	do	+	+	+	+	+	+	+	+	+	+	+	+	+	+
33	42	do	+	+	+	+	+	+	+	+	+	+	+	+	+	+
34	42	do	+	+	+	+	+	+	+	+	+	+	+	----	----	----
35	42	do	+	+	+	+	+	+	+	+	+	+	+	----	----	----

[1] Counting from point of inoculation.
[2] Inoculations were made in young leaves at the top of the plants.
[3] Inoculations were made on small shoots at the base of the main stems.

The plants were kept in the greenhouse and were watched for the appearance of symptoms on the healthy grafts. Symptoms were quite evident when infection occurred, as the curly top virus tends to produce a severe initial shock when introduced into Turkish tobacco. Later, the plants usually recover to a marked degree. In all plants of the first lot, considerable leaf surface was produced on the upper part of the plants in 1 to 2 weeks following placing of the grafts. Plants of the second lot had an appreciable amount of foliage on the upper part of the stem throughout the duration of the tests. Blossom buds were removed as they were formed and at all times the growth was predominantly vegetative.

The results of this experiment (table 4) show that the virus moved downward in a much shorter time than it moved upward. Also, the virus moved upward more slowly in plants that had a leafy top at the time they were inoculated. Seven of these eleven plants showed no symptoms of curly top on the upper graft after an average period of more than 1 year.

TABLE 4.—*Upward and downward movement of virus in stems of Nicotiana glauca 3 feet in height as influenced by presence or absence of foliage at top of plants when inoculated*[1]

Condition of *Nicotiana glauca* stems when inoculated	Position of infected graft	Direction of virus movement	Plants tested	Average time required for appearance of symptoms on healthy graft
			Number	*Days*
No leaves	Top	To root	7	32
	Base	To top	7	130
Leafy tops	Top	To root	11	28
	Base	To top	[2] 11	302

[1] Inoculation made by grafting diseased stems of *Nicotiana tabacum* on *N. glauca* as indicated in table.
[2] 7 of these plants did not show symptoms on the tops until after the tops were defoliated.

EFFECT OF DEFOLIATION AND DARKNESS ON VIRUS MOVEMENT IN TOBACCO

DEFOLIATION

The seven plants, from the experiment just described, that showed no indication of virus in the top grafts after an average period of more than 1 year were used in an experiment to determine whether virus could be made to enter the top graft by upsetting the established food balance by defoliation of the top. The plants were completely defoliated at the top one at a time at intervals until all plants were defoliated. After each plant was defoliated the remaining nondefoliated plants were kept as checks until the defoliated plant showed symptoms of curly top on the new growth. Each defoliated plant had its normal amount of leaf surface on the basal or infected graft. This implies, presumably, that in the production of growth at the top the food reserves of the stem were used first, after which food moved from below, perhaps from the diseased basal shoot and the root system.

The results of these tests are shown in table 5. Symptoms appeared on the new growth of the defoliated tops in an average period of 18 days. This is only slightly longer than the time necessary for the production of enough new growth to show symptoms. No symptoms were evident on any of the top grafts during the time the plants served as checks on defoliated plants.

TABLE 5.—*Effect of top defoliation on movement of virus into the top in plants that had previously restricted the virus to the basal portions for prolonged periods*

Plant no.	Time top graft remained free from virus prior to defoliation of top	Time required for symptoms to appear on top graft following defoliation of top	Plant no.	Time top graft remained free from virus prior to defoliation of top	Time required for symptoms to appear on top graft following defoliation of top
	Days	*Days*		*Days*	*Days*
1	393	18	6	397	15
2	374	29	7	369	11
3	359	11			
4	320	30	Average	368	18
5	365	12			

Although the results from the experiment just described show that defoliation caused the virus to enter previously noninvaded areas, they reveal neither the extent to which the virus had invaded the stems prior to defoliation nor the rate of virus movement into pre-

FIGURE 1.—Type of plants used in studying the upward movement of virus from an infected *Nicotiana tabacum* graft, through a stem of *N. glauca*, to a healthy *N. tabacum* graft at the top of the plant. To cause the virus to move upward, the *N. glauca* plants (left) were defoliated at the top 90 days after inoculation at the base. The plant on the right was not defoliated and was used as a check on the effect of defoliation on upward movement of the virus.

viously noninvaded regions following defoliation. A second experiment was performed to obtain information on these points.

As in previous tests, potted plants of *Nicotiana glauca* were used. These were pruned to a single stem 3 feet long, and a 3-inch segment

from a healthy Turkish tobacco plant was grafted onto the top of each plant. The plants were allowed to grow until mature leaves were present on the healthy graft and also on the lateral shoots of the *N. glauca* stems. A 3-inch segment of stem from a diseased Turkish tobacco plant was then grafted onto the base of each of the *N. glauca* stems. Three months after this latter graft was placed, six of the plants were selected at random and the stems above the infected grafts were cut into 6-inch segments and grafted onto healthy Turkish tobacco plants to determine the presence or absence of virus in the segment and the extent to which the *N. glauca* stems had been invaded during the 3 months the virus had been present in the basal graft. All of the remaining plants, except six that were left for checks at the end of the experiment, were completely defoliated except for the shoots on the infected basal grafts. One of the defoliated plants and one of the check plants are shown in figure 1. At intervals after defoliation, five plants were cut into 6-inch segments and tested for virus by grafting them onto healthy Turkish tobacco plants.

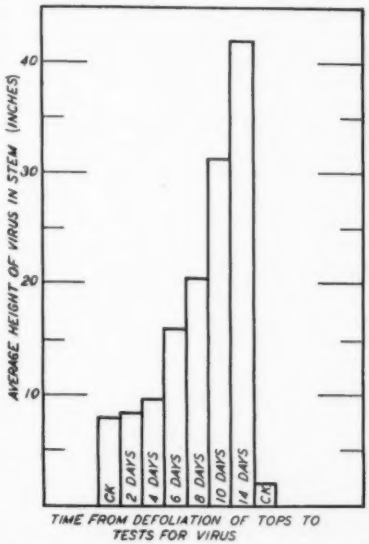

FIGURE 2.—Rate of upward movement of virus in stems of *Nicotiana glauca* following defoliation.

From the results of this experiment (fig. 2), it is clearly evident that the virus moved into the tops of these plants in response to defoliation. The rate of movement apparently was somewhat slower than in *Nicotiana tabacum*, since in a number of instances the virus had moved only a part of the total distance when the stems were cut into segments; whereas, in most instances in *N. tabacum* the virus had either not started toward the opposite end of the plant or had moved the entire distance in a given period. It seems probable also that the upward movement in *N. glauca* stems following defoliation was somewhat slower than the downward movement in stems of plants not defoliated.

There was a distinct difference in the level of virus in the stems of the checks at the beginning of the experiment as compared with the level of virus in the stems of the plants held as checks until the end of the experiment. It may be that this result is not altogether due to chance. The plants were defoliated at the beginning of a period which proved very favorable for growth. Moreover, the infected grafts at the base of the plants used as checks at the end of the experiment were pruned rather severely when the other plants were defoliated. The basal or infected grafts produced abundant growth during the 2 weeks the plants served as checks. Under such conditions, there was probably a very heavy movement of materials toward the base of the plants, and possibly this may have caused a recession in virus level in the stem.

DARKNESS

Two separate experiments were made with Turkish tobacco to determine whether keeping parts of the plant in the dark would induce a more rapid passage of the virus into them than normally occurs in the case of comparable parts in the light.

In the first experiment, plants about 2 feet high were pruned to a height of about 6 inches. Two buds at the top were allowed to develop into shoots. When these shoots were approximately 1 foot tall, the plants were bent so that the stems were in a horizontal position. One of the new shoots which started at the base of the plant after the stem was placed in a horizontal position was inoculated with curly top when it was approximately 1 inch high. Soon after symptoms appeared on the inoculated shoot one of the two larger shoots was placed in the dark, where it remained 7 days. Both large shoots were then cut into 3-inch segments and planted in sand to determine how far the virus had moved into each shoot.

The results indicate that in a few instances the virus moved into the darkened shoot more rapidly than into the shoot kept in the light. However, in some of the plants the virus did not move into either shoot in the time periods used, and in other plants the virus had moved the entire distance to the top of both shoots.

In a second experiment, plants were cut back to a height of about 6 inches, and two shoots were allowed to develop from the upper two buds. When these shoots were about 12 inches high, one shoot on each plant was inoculated with the curly top virus. With 10 plants the noninoculated shoot was placed in the dark, and with 10 other plants the inoculated shoot was placed in the dark. The shoots were kept in the dark 5 days following inoculation of the plants and then were returned to normal light conditions. Checks were used in which both shoots were in the light.

The results of this experiment (table 6) indicate that under the conditions of the experiment darkness had no appreciable effect on virus movement. The virus moved from shoots in the dark to shoots in the light at about the same rate as it moved from shoots in the light to shoots in the dark, and these rates were about the same as movement from one shoot to another in the check plants under normal greenhouse conditions.

TABLE 6.—*Relation of darkness to movement of virus in Turkish tobacco plants having 2 shoots, 1 inoculated and the other not inoculated*

Treatment [1]	Average time for appearance of symptoms on inoculated shoot	Average time for appearance of symptoms on noninoculated shoot
	Days	*Days*
Noninoculated shoot in dark [2]	8.4	11.2
Inoculated shoot in dark [2]	8.8	11.4
Both shoots in light	8.6	11.1

[1] 10 plants were used in each test.
[2] Shoot was kept in dark for 5 days following inoculation.

The experiment just described was repeated, except that shoots were kept in the dark for several days prior to inoculation. However, after these shoots were placed in the dark subsequent growth was limited to a relatively small development at the growing point. It was found that shoots kept in the dark for 10 days or more, even though they were attached to plants having abundant leaf surface in the light, turned yellow, wilted, and died. Therefore, it does not seem probable that a darkened shoot of tobacco will draw its food supply from other shoots of the same plant that are in the light and manufacturing an abundance of carbohydrates. Because of the failure of plants to grow in the dark, no further experiments along this line with tobacco were attempted.

EFFECT OF DEFOLIATION AND DARKNESS ON VIRUS MOVEMENT IN SUGAR BEET

In further experiments on the effect of defoliation and darkness on virus movement, sugar beets were used. The plants were of three kinds and were prepared as follows:

(1) Large beets from the field were topped and placed in pots in the greenhouse. Of the numerous shoots that developed from the crown, three on each plant were selected that were approximately equally spaced about 3 inches apart.

(2) Beets about 1 inch in diameter were taken from pots, defoliated, and split into three parts longitudinally for a distance of 2 inches, the three portions remaining attached at the base. The roots were placed in pots, and the three parts were spread so that the top portions were equally spaced about 3 inches apart. The growth from the tops developed into three separate crowns and in effect three beet plants were obtained, all connected by the basal portion of the root. These beets were of a very susceptible variety.

(3) Large beets were split into three parts longitudinally for a distance of about 6 inches, all three portions remaining attached at the basal end. Each root was placed in a pot and the three parts were spread so that the top portions were equally spaced about 6 inches apart. These plants were of the curly top resistant variety U. S. 1.

DEFOLIATION

After the plants just described had recovered from the pruning and splitting treatment and after three vigorous tops had become established on each plant, several plants were selected and one of the three shoots on each plant was inoculated with the curly top virus by means of leafhoppers. At the time of inoculation one of the noninoculated shoots was defoliated except for the very young leaves less than 1 inch long. The third shoot on each plant served as a check on the effect of defoliation on the movement of virus from the inoculated shoot into other parts of the plant

As shown in table 7, defoliation resulted in a very rapid movement of virus into the defoliated shoots and in the production of symptoms on the new growth from these shoots in average periods of 10.6, 10.8, and 12.8 days following inoculation of the plants. That defoliation was responsible for this movement of virus is proved quite conclusively by the fact that the check shoots, not inoculated and not defoliated, remained free from symptoms an average of 143, 108, and 110 days,

respectively, for the different types of beets. A typical plant of the resistant variety U. S. 1, photographed 186 days after inoculation and defoliation, is shown in figure 3.

FIGURE 3.—Effect of defoliation on virus movement in beet. One shoot (left) was inoculated, one shoot (right) was defoliated, and one shoot (middle) was retained as a check. Symptoms of curly top appeared on the inoculated shoot and on the defoliated shoot 12 and 15 days, respectively, after inoculation. The check shoot was free from symptoms after 186 days.

TABLE 7.—*Effect of defoliation and darkness on the movement of virus into noninoculated shoots of beet plants*

Type of beet [1]	Treatment of test shoot	Plants inoculated	Average time for appearance of symptoms on—		
			Inoculated shoots	Treated shoots	Check shoots
		Number	*Days*	*Days*	*Days*
Large beets with 3 shoots	Defoliated	5	9.4	10.6	143
Susceptible	do	6	9.8	10.8	108
Resistant	do	6	12.5	12.8	110
Large beets with 3 shoots	Darkened [2]	3	12.0	17.6	55
Susceptible	do	6	10.2	12.1	102
Resistant	do	12	13.5	16.6	183

[1] Large field beets were topped and 3 crowns allowed to form on each plant. The susceptible and resistant beets were split longitudinally into 3 portions which were left connected at the base, 3 separate crowns being produced on each root.

[2] Shoots were in dark for 5 days following inoculation.

DARKNESS

Plants of the same types as those used in the defoliation tests just described were used in experiments to determine the effect of a period of darkness on the movement of virus into one of the noninoculated shoots. In these tests, one of the three shoots of each plant was inoculated by means of leafhoppers. When the plants were inoculated they were placed with one of the noninoculated shoots in the

dark and the other noninoculated shoot and the inoculated shoot in the light. One shoot was kept in the dark 5 days. The plants were then returned to normal light conditions.

Without exception, the new growth of the shoots subjected to darkness produced typical curly top symptoms. A typical plant of the resistant variety U. S. 1 in this series, photographed 90 days after inoculation, is shown in figure 4, and a plant of a susceptible variety, photographed 48 days after inoculation, is shown in figure 5. As shown in table 7, the average time required for the production of symptoms on the darkened shoot was not much longer than the

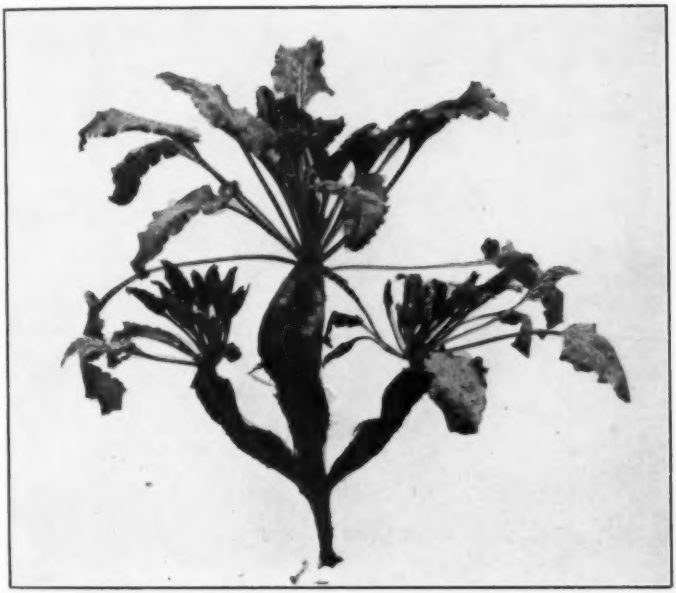

FIGURE 4.—Effect of darkness on the movement of virus in resistant beets. One shoot (left) was inoculated, one shoot (right) remained in the dark 5 days, and one shoot (middle) was kept as a check. Symptoms of curly top appeared on the inoculated shoot and on the darkened shoot 11 and 13 days, respectively, after inoculation. The check shoot was free from symptoms when the plant was removed from the pot and photographed 90 days after the plant was inoculated.

average time required for production of symptoms on the inoculated shoot. The check shoots remained free from symptoms for periods varying from 30 days to more than a year and averaged 55, 102, and 183 days, respectively, for the different types of plants used.

After the check shoots of some of the plants of this experiment had remained free from symptoms for periods ranging from 3 to 9 months, seven of the plants were selected for a test to determine whether a movement of virus to the check shoot could still be induced. Preceding these tests, nonviruliferous leafhoppers were fed on the young leaves of each of the three shoots of each plant, and then caged singly on seedling beets. No virus was obtained from any of the check shoots, whereas virus was obtained from all of the other shoots.

This test indicated an absence of virus from the check shoots and an abundance of virus in the other shoots at the beginning of the test.

To provide as much stimulus as possible for food movement, the healthy shoots were defoliated before being placed in the dark. After 5 days in the dark, the plants were returned to the normal conditions of the greenhouse. Symptoms appeared on all of the shoots that had been kept in the dark. The time required for appearance of symptoms following treatment varied from 6 to 12 days.

FIGURE 5.—Effect of darkness on the movement of virus in a susceptible beet. One shoot (left) was inoculated, one shoot (right) was kept in the dark 5 days, and one shoot (middle) was retained as a check. Symptoms of curly top appeared on the inoculated shoot and on the darkened shoot 8 and 11 days, respectively, after inoculation. The check shoot was free from symptoms when the plant was removed from the pot and photographed 48 days after the plant was inoculated.

RATE OF MOVEMENT OF VIRUS INTO DARKENED SHOOTS

After it was determined that the virus moved into all shoots kept in the dark for 5 days, tests were made to determine the shortest interval in darkness required to cause movement of virus into a darkened shoot. Large plants split to produce three crowns as described were selected. One shoot was inoculated with curly top. After symptoms appeared on the inoculated shoot, one of the remaining shoots of each of five plants was placed in the dark. Plants were returned to normal light conditions at 24-hour intervals for 5 days.

The results of two repetitions of this test are shown in table 8. It is evident from these results that a relatively short exposure to darkness was sufficient to cause the movement of virus from diseased shoots into darkened noninoculated shoots that had previously

remained free from disease for relatively long periods when under normal conditions for growth.

TABLE 8.—*Effect of periods of darkness on movement of virus from diseased to healthy shoots in beet plants having 3 shoots*

Type of beets	Time for appearance of symptoms on inoculated shoot	Time noninoculated shoots remained healthy prior to placing 1 shoot in the dark	Time 1 noninoculated shoot was in the dark	Time for appearance of symptoms on darkened shoot after being placed in dark	Time check shoot remained healthy after 1 noninoculated shoot was placed in dark
	Days	*Days*	*Days*	*Days*	*Days*
Large resistant beets split to produce 3 crowns	12	79	1	151	80
	7	79	2	3	179
	15	63	3	5	142
	21	63	4	7	131
	24	63	5	9	196
Large susceptible beets with 3 shoots	14	16	1	5	30
	15	16	2	5	26
	13	16	3	5	34
	14	16	4	5	47
	16	16	5	6	113

In further tests leafhoppers were used to determine if the virus was present in the different shoots. Plants having three shoots, one of which had distinct curly top symptoms, were used. Nonviruliferous leafhoppers were fed 1 day on the small leaves of each shoot and then caged singly on seedling beets. These tests in each case showed that the virus was present in the inoculated shoot and absent from the other two. All of the leaves except those less than 1 inch long were removed from one of the noninoculated shoots. Nonviruliferous leafhoppers were caged on the remaining small leaves and also on the small leaves of the other two shoots. The shoot that was defoliated was then placed in the dark. At 24-hour intervals for 4 days, the leafhoppers were replaced by other nonviruliferous leafhoppers. Those removed were caged singly on seedling beets to determine whether they had picked up virus.

Five plants were tested in this manner. In one instance the leafhoppers picked up virus from the darkened defoliated shoot during the first 24-hour period and in all instances they picked up virus during the second 24-hour period and in every 24-hour period thereafter for 4 days. The leafhoppers also obtained virus from the inoculated shoot, but no virus was obtained from the noninoculated shoot that remained in the light. The check shoots were free from symptoms 3 months after the plants were inoculated.

RETENTION OF VIRUS IN ETIOLATED LEAVES

If movement of virus is correlated with food translocation, it seems probable that introduction of virus into the distal end of an etiolated leaf should result in a relatively slow invasion of the leaf tissue. It should be expected that the virus would be retained in the etiolated leaf for a prolonged period, since in such a leaf movement of materials would be into the leaf from the crown and presumably there would be very little, if any, movement of materials in the opposite direction.

Since the beet has a large quantity of stored food and since it will grow readily in the dark, it was used in testing this hypothesis.

The tops were removed from potted plants having roots 2 to 4 inches in diameter. The defoliated plants were placed in a ventilated dark box and left until leaves more than 6 inches long were produced (fig. 6). Inoculation was effected by caging 10 viruliferous leafhoppers on one leaf of each plant for a period of 48 hours. Following inoculation the plants were removed from the dark box at intervals up to 21 days. At the time the plants were returned to normal light conditions, the inoculated leaves were removed from half of the plants.

FIGURE 6.—Type of etiolated plant used in studying the movement of virus out of etiolated leaves. Virus moved out of green leaves in 4 hours or less and was retained in etiolated leaves 21 days in some instances.

The remaining plants, from which the inoculated leaves were not removed, were used as checks on the amount of infection produced by the leafhoppers.

Although it was known that the virus moved inward very rapidly when introduced into the distal end of green leaves, a further check was set up in which 10 viruliferous leafhoppers were caged on the distal end of one leaf of each of 20 plants. After 4 hours the leafhoppers were removed, and the inoculated leaves were severed from half of the plants 6 inches from the point of inoculation. The remaining plants were retained as checks, from which the inoculated leaves were not removed.

The results of this experiment are shown in table 9. The removal of the inoculated leaves from the normal green plants 4 hours after the leafhoppers were placed on them had little or no influence on the number of plants that developed curly top. Infection occurred and the virus moved out of the inoculated leaf in 4 hours. On the other hand, the virus was retained in the inoculated leaves of the etiolated plants until after they were returned to the light, except in one plant of the 10-day series and in three plants of the 21-day series. It should be mentioned that in the case of the four etiolated plants, in which the virus passed into the crown while the plants were in the dark, the inoculated leaves were partially dead when they were returned to the light. It is possible that in such leaves there may have been an inward movement of materials derived from disintegrated protoplasm even though no carbohydrates were being synthesized.

TABLE 9.—*Retention of virus in etiolated beet leaves*

Time leafhoppers were on plants	Time plants were in dark	Inoculated leaf removed when plant was returned to light				Inoculated leaf not removed			
		Plants inoculated	Plants infected	Average incubation period of disease from—		Plants inoculated	Plants infected	Average incubation period of disease from—	
				Inoculation	Return to light			Inoculation	Return to light
	Days	*Number*	*Number*	*Days*	*Days*	*Number*	*Number*	*Days*	*Days*
4 hours	[1] 0	10	4	13.7		10	5	15.0	
2 days	2	10	0			10	8	11.5	9.5
	5	14	0			15	14	11.1	6.1
	8	8	0			8	6	13.0	5.0
	10	8	1	17	7	8	8	15.0	5.0
	14	8	0			8	8	18.7	4.7
	21	8	3	26	5	7	7	25.0	4.0

[1] Green plants, not placed in dark; the inoculated leaf was removed from 10 of the 20 plants 4 hours after the leafhoppers started feeding.

In the 10-, 14-, and 21-day series, the inoculated leaves that were removed upon return of the plants to the light were divided into blades and petioles, and nonviruliferous leafhoppers were allowed to feed on the two parts 2 to 4 days. The leafhoppers were then caged on healthy seedling beets. Virus was recovered from 23 of 24 blades tested, but from only 2 of 24 petioles tested. One of the petioles that yielded virus was from a plant in which the virus had passed out of the inoculated leaf before the plants were returned to the light, and one was from a plant in which the virus had not passed out of the inoculated leaf into the crown at least in quantity to produce disease.

This evidence indicates that the virus multiplied locally in the leaf blade and that in most instances it was restricted to the blade during the time the plants were in the dark.

Further evidence of multiplication and localization in the blade is furnished by the incubation periods of the disease shown in table 9. Counting from the time the plants were inoculated, the incubation period in the etiolated plants varied from 11.1 to 25 days. However, counting from the time the plants were returned to the light, the incubation period tended to decrease as the time in the dark increased up to and including the 5-day exposure to darkness, after which it remained in the range of 4 to 5 days. This is a strikingly short incu-

bation period for the disease in large beets, as indicated by the incubation period of 13.1 to 15 days in normal green plants. It seems probable that, following infection of beet plants by means of leafhoppers, 10 to 15 days may be required for the virus to increase to a concentration sufficient for the production of symptoms. It is probable that the virus multiplied in the distal ends of the etiolated leaves during the time the leaves were in the dark and that when the plants were returned to the light virus was present in sufficient concentration to produce symptoms. Therefore, the production of symptoms was dependent only on movement of virus to the growing point and on the production of enough leaf growth to express symptoms. Apparently the time required for the transport and growth is in the neighborhood of 4 to 5 days.

Tests were made to determine the time etiolated leaves must remain in the light before virus is transported from them to the crown. In these tests, plants of the type already described were used. All of the larger leaves were removed and the plants were kept in the dark until etiolated leaves 6 inches long were available for inoculation. The plants were inoculated by leafhoppers that were allowed to feed 2 days on the distal end of one leaf of each plant. The plants remained in the dark 5 days from the beginning of inoculation. They were then returned to the light and the inoculated leaves were removed at intervals of 1 day over a 3-day period.

The results of this experiment are shown in table 10. The virus passed out of the inoculated leaf in all instances in 3 days and in some instances in 24 hours after the plants were returned to the light. The incubation period, counting from the time the plants were returned to the light, generally decreased as the period for virus movement out of the inoculated leaf increased. Possibly in plants in which the inoculated leaf was retained 1 and 2 days after return to light, only a small portion of the virus in the inoculated leaf had moved into the crown before the leaf was removed. Therefore, before symptoms could be produced, time was required for increase of virus to a concentration sufficient to cause symptoms; whereas, in the check plants and in the plants on which the inoculated leaf remained 3 days, the virus may have migrated from the inoculated leaf in quantity to cause symptoms as soon as growth was sufficient to express symptoms.

TABLE 10.—*Time required for virus to move out of inoculated leaves of etiolated plants kept in the dark 5 days after inoculation and then returned to the light*

Time in light before inoculated leaf was removed	Plants inoculated	Plants infected	Period of incubation of disease in plants from—	
			Inoculation	Return to light
	Number	*Number*	*Days*	*Days*
0	12	0		
1 day	11	2	16.0	11.0
2 days	12	6	14.1	9.1
3 days	12	12	10.5	5.5
Check [1]	9	9	10.7	5.7

[1] Inoculated leaf not removed.

DISCUSSION

It is quite obvious that under certain conditions movement of the curly top virus in sugar beet and tobacco can be influenced and to a considerable degree controlled by manipulations that presumably influence food-concentration gradients. This is particularly striking in the case of sugar beet. In fact beet seems to be an unusually favorable plant in which to study correlation between virus movement and food transport. Its ability to produce abundant growth in the absence of light shows that its food supply is relatively mobile and that food reserves of the root are readily available for top growth. Also, continuous development of shoots in the dark over long periods indicates that these shoots may obtain a portion of their food supply from other shoots of the same plant in the light.

Relative upward and downward movement of the virus in stems of certain types of plants of *Nicotiana tabacum* and results from defoliated and nondefoliated plants of *N. glauca* furnish additional evidence indicating a relation between virus movement and food transport.

Apparently in *Nicotiana tabacum* darkness neither measurably accelerated nor retarded movement of the virus. This is substantially in agreement with results obtained by Caldwell (*5*), who used the virus of tobacco mosaic. Failure of darkness to affect measurably the movement of the virus in tobacco, however, cannot be accepted as proof that food transport does not influence virus movement, for there is strong evidence that darkness does not influence food movement in tobacco to nearly the same extent that it does in such plants as sugar beet.

Darkened tobacco shoots produced very little growth after being deprived of light and began to die in a few days after being placed in the dark. Thus darkened shoots are probably unable to draw appreciable amounts of food from either food reserves or food synthesized by shoots in the light. Curtis (*9*) suggests that transport of materials from leaves that are approaching death or that are about to abscise may be strictly unidirectional and outward only. Under conditions of darkness the uncertainty regarding the direction of movement of food materials in tobacco is so great that any correlation that may exist between food movement and virus movement would be difficult to detect.

When virus was introduced into the tips of mature leaves of normal beets it moved away from the point of introduction a distance of approximately 10 inches in 4 hours, whereas with etiolated plants in most instances it did not move out of the inoculated leaf in 14 to 21 days. The movement out of green leaves was probably much faster than is indicated by these results, for other tests designed to measure more accurately the speed of virus movement (*3*) have shown that when introduced into the distal end of a beet leaf by an individual leafhopper the virus moved out of the leaf a distance of 6 inches during the first 6 minutes of leafhopper feeding. After the virus moved out of a mature inoculated leaf and passed into the crown and root, where it presumably soon reached a maximum concentration, it moved into mature noninoculated leaves very slowly. This indicates that virus concentration has very little measurable influence on virus movement. This is supported by evidence (*4*) indicating that beet seeds are able to accumulate virus against a virus gradient.

The curly top virus probably produces infection only when it is introduced into the phloem, and it appears to be closely limited to the phloem tissue even after becoming thoroughly distributed to all parts of the plant. Therefore, it seems safe to assume that the factors involved in the virus movements that have been studied are concerned with transport of materials in the phloem.

It seems rather definitely established by these results that the curly top virus moves rapidly and unidirectionally and that the movement occurs in the phloem and is correlated with food transport. In view of these considerations, the virus probably moves passively through the plant in response to the action of definite physiological processes that take place regularly in normal plants. Virus movement then may represent a measurable response to the operation of mechanisms concerned with the transport of organic materials in general.

Among plant physiologists there is still much disagreement as to the manner of transport of organic materials, although it is agreed rather generally that the mechanism involved must provide for rapid transport of materials in the phloem.

Went (*20*) suggested that growth hormones move cataphoretically toward growing points along an electrical potential. It is conceivable that virus particles might bear a charge that would cause them to move in a given direction (downward from the tip of a beet leaf, for example) at a rapid rate. However, if such a force is responsible for virus movement it must be further assumed, in the light of evidence, that defoliating or darkening a part causes either a reversal of charge on the virus particles or a reversal of electrical potential along the path of movement to permit the passage of virus into shoots into which it would not move under normal conditions of light and growth. It is, of course, conceivable that such reversals may occur, but no evidence supporting such a contention is available.

Van den Honert (*13*) suggested movement of food materials along interfaces as a result of surface-tension changes in the protoplasm. As has been pointed out (*14*), it is difficult to estimate the rate of movement of materials to be expected as a result of surface forces and, moreover, the physical requirements for the operation of such a system are apparently not provided in the phloem. Since this system does not provide for unidirectional movement, it does not seem probable that it functions in the movement of viruses.

A theory of food movement long held and recently given special emphasis by Curtis (*9*) suggests that food materials move by diffusion, aided by protoplasmic streaming. The greatest rate of movement attainable by such agents, even if it be assumed that there is streaming of material in the mature sieve tube (which has not been observed), seems inadequate to account for the translocation of food materials, and it seems certain that it does not provide for the rapid movement observed with viruses. Moreover, such a mechanism would cause the virus to move upward and downward in a stem at approximately equal rates, and defoliation or darkness would not greatly accelerate movement of the virus into defoliated or darkened parts.

Mason, Maskell, and Phillis (*14*), recognizing the inadequacy of simple diffusion aided by protoplasmic streaming to account for the rapid movement of materials observed in the cotton plant, suggested that a mechanism may exist in the phloem that enormously accelerates diffusion through expenditure of energy on the part of the plant.

The nature of this hypothetical mechanism is very obscure. Such a mechanism, even if it provided for the rates of virus movement detected, would not account for the directional movements observed. To account for the observed virus movement by this mechanism it would be necessary to assume that the virus particles are influenced in some manner by molecules of some food compound and move along the concentration gradients of the influencing compound.

The theory of food translocation which seems best to fit the observed facts of virus movement is that of mass movement of materials in the phloem in a manner similar to that suggested by Münch (*15*) and by Crafts (*7*). This theory assumes that products of photosynthesis pass from parenchyma into the phloem of the synthesizing regions of the leaf and increase the osmotic pressure of the phloem of these regions. This in turn causes water to move into the phloem, thus setting up a higher hydrostatic pressure. This pressure causes a movement of materials through the phloem to regions where the pressure is lower.

Steward and Priestley (*18*) and Curtis (*9*) have advanced objections to this theory, based principally on the resistance that the phloem would presumably offer to the mass movement of liquid contents. Crafts (*8*) has met this objection, at least in part, with experimental evidence that indicates that the mature sieve tubes become completely permeable to dissolved substances, so that the sieve-tube lumina are probably the principal channels of movement. Resistance under this condition would be largely restricted to the sieve plates. Esau (*10*) has strengthened this view by showing that symptoms of curly top appear in the phloem only in regions having mature sieve tubes.

The recent observations on phloem exudation (*8*), showing that this process may continue for periods of 24 hours in certain plants, indicate that a mass flow actually occurs in the phloem and that volumes of solution equivalent to the space occupied by the phloem in many centimeters of stem may be delivered at the cut end, provided the channels are kept open. In this connection it is interesting to remember that phloem exudate from curly top infected beets contains virus in high concentration even after the beets have exuded phloem content intermittently over a period of several days.

A mass movement of food materials through the plant in accordance with the requirements of the mass-flow theory would produce a distribution of virus throughout the phloem network similar to that which has been observed. Virus introduced into the phloem of the distal end of a beet leaf actively synthesizing and transporting food would move toward the regions of food storage and utilization at the rate at which liquids in the phloem were moving, assuming no selective interference. Multiplication would affect the concentration of virus, but its effects on movement would not be measurable. After the virus reached the regions of food storage and rapid utilization, it would tend not to move into mature leaves, since in such leaves movement of materials in the phloem would be toward the region of storage and growth and counter to the available path for virus movement.

Likewise, in a beet having three shoots, if one were infected virus would move to the growing point and into the storage region below but would tend not to move into the other two shoots if their photosynthetic areas were sufficient to keep constantly a high pressure in the

phloem. However, if one of these two shoots were placed in the dark the normal conditions would be upset. No carbohydrates would be synthesized and food would be used for growth, thus lowering the osmotic concentration and hydrostatic pressure in the phloem. This would permit materials to move into the darkened shoot from areas having higher pressures, which, in the case under consideration, would be one shoot free from virus and one shoot laden with virus. Under such conditions, it would be expected that the shoot in the dark would receive virus carried into it along with food materials from the originally infected shoot. This expectation seems to be fully realized in the experiments that have been described.

It seems highly probable that the curly top virus can be used as an indicator of food translocation. At least, the evidence points toward this conclusion so strongly that extensive exploration of the possibilities inherent in the question seems warranted. It seems probable also that viruses in general move through affected plants in response to the operation of factors responsible for food translocation.

SUMMARY

The time required for invasion of a beet plant by the curly top virus varies somewhat with the size of the plant. Seedling plants are probably almost completely invaded in 2 to 4 days. In some instances in larger plants more than 4 weeks were required for virus to pass from a mature inoculated leaf to a mature noninoculated leaf on the opposite side of the plant.

In Turkish tobacco, *Nicotiana tabacum*, the virus moved downward toward the root system more rapidly than in the reverse direction. In two experiments with *N. glauca*, the virus moved downward a distance of 3 feet from infected Turkish tobacco grafts placed at the top and produced symptoms on healthy grafts at the base in average periods of 28 and 32 days. When the positions of the diseased and healthy Turkish tobacco grafts were reversed, the virus required averages of 130 and 302 days to move upward and cause symptoms on the top grafts. In some of these plants the virus did not reach the tops in an average time of more than 1 year. However, when these latter plants were defoliated at the top the virus moved into the top grafts and produced symptoms in an average period of 18 days following defoliation.

Other tests showed that the virus moved only a short distance from the bottom infected graft in 3 months in *N. glauca* plants having an abundance of foliage on the tops. After defoliation of the tops, the virus invaded the upper part of the plants in 14 days or less.

When one shoot of a beet plant having three shoots was infected with curly top and a second shoot of the same plant was defoliated or placed in the dark for 5 days following inoculation, the virus passed into the defoliated or darkened shoot and produced symptoms in average periods of 10.6 to 17.6 days, which were 0.1 to 5.6 days longer than the average periods for the productions of symptoms on the inoculated shoot. The third or check shoot remained free from symptoms average periods of 55 to 183 days. Other tests in which leafhoppers were used to detect the presence of virus showed that virus moved from inoculated shoots showing symptoms into noninoculated shoots that were both darkened and defoliated, in 24 to 48 hours.

When virus was introduced into the distal end of etiolated beet leaves in the dark it failed to move out of the inoculated leaf in most instances in periods up to and including 21 days, although it moved out of green leaves in the light in 4 hours. After etiolated leaves had retained virus 5 days, the virus moved out of the inoculated leaf in 24 to 72 hours following return of the plants to the light.

The way in which the curly top virus invades the plant indicates that virus movement bears little or no relation to virus multiplication or to virus concentration gradients, but is dependent on physiological processes that take place in the normal plant.

It is apparent that in both beet and tobacco the virus can be prevented from entering certain parts by providing conditions for the synthesis of an excess of carbohydrates and that its passage into certain parts can be induced by providing a food deficit. This evidence strongly indicates that the movement of the virus is correlated with food transport and suggests that the virus may be used as an indicator of food translocation. If virus is a true indicator of food translocation, its behavior in the plant strongly supports the theory of mass movement of materials in the phloem.

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A HUMIDITY- AND TEMPERATURE-CONTROL CABINET FOR GROWING PLANTS [1]

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INTRODUCTION

The demand for experimental evidence on many problems requiring an accurate control of temperature and humidity prompted the construction of an apparatus in which growing plants could be placed under as nearly natural light as could be obtained under controlled conditions. In view of the fact that only the visible region of the solar spectrum and the near ultraviolet rays are used in the process of photosynthesis, as reported by Arthur (*1*) [2] and substantiated in the review by Popp and Brown (*5*), it seemed desirable to use glass in the construction, since the use of natural light would make a more nearly normal condition for plant development. Window glass transmits 90 to 95 percent of the total visible radiation of sunlight and absorbs only the extreme infrared and the ultraviolet beyond about 320 mμ. Neither of the regions absorbed appears to be of any appreciable significance as far as plant growth is concerned.

Cabinets to control humidity and temperature have been designed for use in laboratory experiments, but most of them are elaborate and expensive. Chace (*3*) and Carson (*2*) described methods of controlling humidity in small spaces by means of aqueous solutions of sulphuric acid. A feature of the cabinet designed by the present writers that was not used in those described in the literature is the utilization of sunlight instead of artificial light. This, however, made it more difficult to control the temperature, because of heat accumulation from the rays of the sun that penetrated the glass. Wilson (*7*) showed, however, that the relative humidity over any concentration of sulphuric acid is practically independent of the temperature. As pointed out in a previous paper by the present writers (*8*) the use of sulphuric acid is a very satisfactory means of controlling the relative humidity under similar conditions.

DESCRIPTION OF THE CABINET

A view of the cabinet designed by the writers is shown in figure 1. It is a double chamber constructed entirely of window glass except the floor and framework. The glass is sealed to the frame and floor, making it practically airtight. The mechanical equipment is enclosed below the chamber and fastened to the top of the refrigerator, on which the cabinet is constructed and from which cold air is used to aid in the temperature control. While the cabinet is a separate unit

[1] Received for publication July 28, 1936; issued May 1937. Cooperative investigations of the Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Kansas Agricultural Experiment Station. Contribution no. 262, Department of Agronomy, and no. 71, Department of Agricultural Engineering, Kansas State College of Agriculture and Applied Science.

[2] Reference is made by number (italic) to Literature Cited, p. 507.

it was mounted above a low-temperature chamber (*6*) in order to have a supply of cold air for temperature control and dehumidification. Details of the construction and arrangement of the controls and mechanical equipment are shown in figure 2.

RELATIVE HUMIDITY CONTROL

Control of relative humidity is brought about by using an aqueous solution of sulphuric acid in the inside chamber. As shown in figure 2, the acid container, which is a lead trough, is built around the temperature controls, instrument stand, and the openings in the floor through which the plants are introduced into the chamber. The air-circulating fan is located in such a position that a current of air blows over

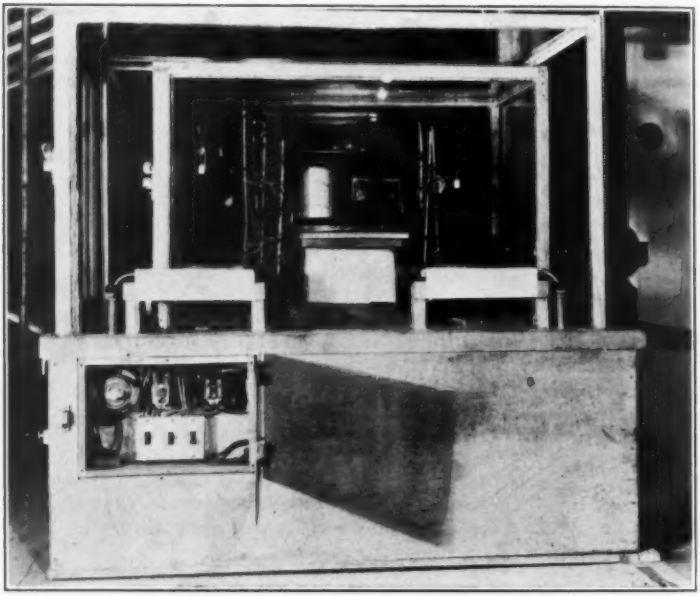

FIGURE 1.—Humidity- and temperature-control cabinet.

the acid on one side, agitates the surface, and causes a continuous flow of acid through the trough. This fan runs continuously, as it is used only for circulating air.

Different percentages of relative humidity are obtained by using different concentrations of sulphuric acid, since the amount of water evaporated varies with the concentration of the solution. Wilson (*7*) pointed out the advantages of this method for obtaining any desired relative humidity and worked out the physical-chemical theory of the calculations.

The cabinet is also equipped with a humidostat used for dehumidifying. Dehumidification is brought about by pumping dry air out of the low-temperature chamber into the cabinet. This was found necessary because of the moisture transpired by the plants. A rotary air pump with a capacity of 4.7 cubic feet per minute, driven by a motor, is used for this purpose.

TEMPERATURE CONTROL

The temperature is controlled entirely by radiation through the glass into the inner chamber. Two thermoregulators, one for the cold air and one for the heaters, are located in the inner chamber. No conditioned air for temperature control is introduced into the inner chamber, but as the temperature changes in this chamber, switches are thrown automatically to turn on or off either cold or hot air that is introduced between the chambers. This will raise or lower the temperature in the inside chamber, as the case may be, by radiation through the glass of the inside chamber. The thermoregulators are sensitive to 1-degree change in either direction and are

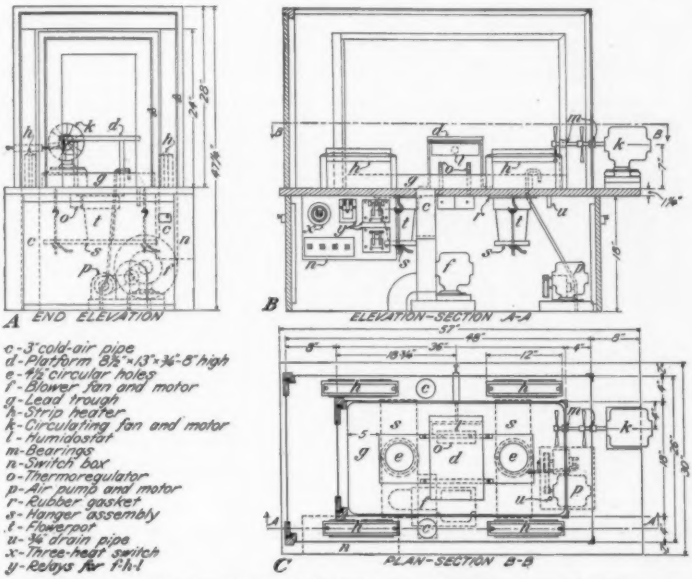

FIGURE 2.—Details of construction of the humidity- and temperature-control cabinet.

regulated to turn on the cold-air fan or the heaters as the temperature of the inner chamber fluctuates. The outer chamber is constructed around the inner chamber, with a 4-inch space allowed around three sides and the top and an 8-inch space in the end containing the door. Mounted on the floor of this intervening space are four 250-watt strip heaters hooked to a 3-way switch to supply 250, 500, or 1,000 watts. A blower-type fan mounted on a ¼-horsepower electric motor draws the cold air out of the refrigerator and blows it up into the intervening space. The air is brought in on one side and back to the refrigerator on the other. The connections are of 3-inch galvanized-tin pipes. In order to obtain a rapid distribution of the heat, as well as of the cold air, between the cabinets, and also to have a good circulation of air in the inner chamber, a ⅛-horsepower motor is mounted on the floor at one end outside of the cabinet and connected to a shaft on which are two fans, one in the inner chamber and one in the space

between the chambers. The shaft is mounted 7 inches above the floor through 1-inch holes in the glass. Bearings placed in rubber stoppers hold the shaft in place.

DISCUSSION

The specific gravity of the acid used in a closed chamber is probably as accurate a measure of the relative humidity of the circulated air as any instrument devised for that purpose. In the operation of this cabinet the acid solution is placed in the cabinet and a few hours are allowed for the relative humidity to become stabilized. The recording hygrothermograph then is adjusted according to the graph worked out by Wilson (7) from the density of the acid. This may be checked at intervals by a hygrometer and psychrometric tables (4).

The cabinet is designed so that only the plants themselves are in the controlled temperature and humidity chamber. The pots are placed

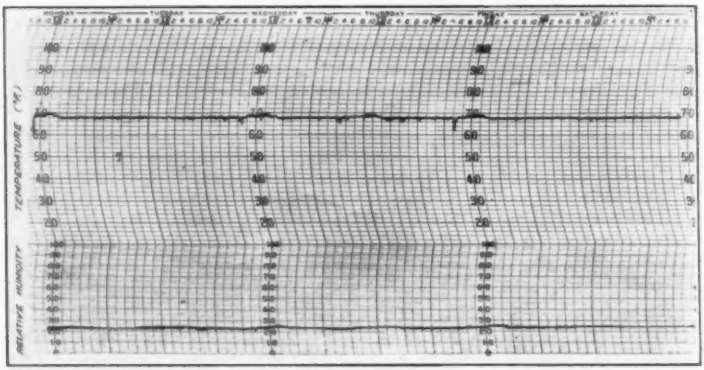

FIGURE 3.—Hygrothermographic record from the humidity- and temperature-control cabinet from Jan. 27 to Feb. 1, inclusive.

below, as shown in figure 2, *t*. There are two holes 4½ inches in diameter in the bottom of the chamber, through which the plants are introduced, and the pots are clamped tightly against the bottom and sealed by a rubber gasket. The clamps also hold a pan under the pot for watering. The trials have been confined to alfalfa plants, but by varying the method of sealing the pot almost any type of plant might be grown. Before a plant is placed in the chamber the soil in the pot is removed from around the crown and a rubber collar is tied tightly around the taproot and allowed to extend above the level of the soil. The soil is then replaced and the pot is sealed with paraffin. After the pot is sealed in this manner no moisture can get into the cabinet except that which the plant transpires.

After numerous trials it was found that during the day the moisture transpired by the plants was not absorbed by the acid as rapidly as the plants gave it off, and thereby the relative humidity was raised. Therefore, in order to maintain a constant humidity during night and day, dehumidification was necessary in the daytime, as previously mentioned. At night the humidity will reach the level determined by the specific gravity of the sulphuric acid.

Soil moisture is also a factor that may be controlled and used in conjunction with this chamber. With the proper set-up of plant containers and by the use of a balance the plants may be lowered onto the balance momentarily and brought back to weight by the addition of water through an opening in the paraffin seal. It has been noted that if the air-circulating fan is stopped very little change in temperature or humidity will occur when the holes through which the plants are placed are opened for a few minutes. The weighing and bringing up to weight can be done without entirely removing the plant from the chamber.

Figure 3 is a hygrothermographic record, covering a 6-day period, which shows the regulation of humidity and temperature obtained. The relative humidity as recorded on the chart indicates that the acid was diluted slightly by the water transpired by the plants. A check, made with acid hygrometers, showed this to be the case. However, such dilution may be balanced by adding daily a predetermined volume of concentrated acid. Other charts have been obtained at different temperatures ranging from 65° to 110° F. and with relative humidities ranging from 0 to 90 percent with just as good regulation in all cases except where the wet-bulb depressions are low. In that case the regulation is constant but the humidity shows a range of 2 percent owing to the condensation of some of the moisture on the glass, which is chilled as the cold air comes on. While the dehumidifier aids in keeping a constant humidity by quickly drying up the condensation on the glass, the lag of the instrument shows a somewhat wider fluctuation than actually occurs around the plants. Plants naturally transpire more rapidly in a dry atmosphere than in a wet one, so that the dehumidifier operates at longer intervals with the lower humidities than with the high humidities in use.

SUMMARY

A cabinet for the control of humidity and temperature, for use in agronomic experiments with plants growing under glass and exposed to natural light, is described.

Relative humidity is controlled by using an aqueous solution of sulphuric acid in the inner chamber of the cabinet.

Temperature is controlled entirely by radiation through the glass into the inner chamber.

The use of the cabinet may be extended to studies with soil-moisture control, thus providing equipment controlling three environmental factors that profoundly influence plant development. At the same time the plants are held under as nearly normal light conditions as it is possible to obtain under glass.

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VARIATION IN MASS ISOLATES AND MONOCONIDIUM PROGENIES OF CERATOSTOMELLA ULMI[1]

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INTRODUCTION

There are in the United States three recognized vascular diseases of elms, namely, the Dutch elm disease, cephalosporium wilt and dieback (*10*, *16*),[3] and verticillium wilt (*20*). Externally all are characterized by symptoms typical of wilt diseases in general, and the discolorations developed in the vascular systems of trees affected by the three diseases cannot be differentiated with certainty by observation. Moreover, one tree may sometimes be infected with two or more organisms.

At present the only method of accurately diagnosing cases of the Dutch elm disease is by culturing specimens from suspected trees. Obviously it is important to have as complete information as possible on the limits and possibilities of variation of *Ceratostomella ulmi* (Schwarz) Buisman in culture.

Buisman (*4*) and Swingle (*19*) have demonstrated that *Ceratostomella ulmi* is normally heterothallic, and Swingle has found that monoascospore progenies comprise a rather wide range of cultural races. Swingle briefly mentioned that monoconidium and monoascospore cultures produce, by sectoring, variants strikingly different from their parental types.

This paper is an account of (1) the range in variation thus far noted in the cultural characters of mass isolates of *Ceratostomella ulmi*, (2) the appearance of sector variants in some of these mass isolates, and (3) the sectoring of monoconidium progenies of some of the variant-producing isolates. In lieu of lengthy descriptions, photographs are presented to show the strikingly different natures of some of the cultures studied. These observations and experiments are reported at this time because it is considered that they may be of service to those newly concerned with the culturing of specimens and identification of organisms isolated from suspected cases of Dutch elm disease.

MATERIALS AND METHODS

Thousands of cultures of *Ceratostomella ulmi*, made from various types of source material but especially from specimens submitted for cultural diagnosis, have been available for observation and study. A few of these cultures have been retained for special observations and comparisons because they represented departures from the typical isolate in one or more features.

[1] Received for publication Aug. 17, 1936; issued May 1937.

[2] The writer wishes to acknowledge his indebtedness to Curtis May, senior pathologist, Division of Forest Pathology, for many helpful suggestions and criticisms. He also wishes to make special acknowledgment to T. W. Graham, junior forest pathologist, Emergency Conservation Work, for the photography in connection with this paper.

[3] Reference is made by number (italic) to Literature Cited, p. 522.

For comparisons the isolates were grown on potato-sucrose agar (potato decoction + sucrose, 2 percent, and agar, 1.8 percent, acidified to approximately pH 4.6), and malt agar (malt extract, 2 percent, and agar, 2 percent, approximately pH 5.5), media that are unquestionably relatively rich in available nutrients. Convenience and the need for immediate practical application of the observations were the principal reasons for the use of these media in the experiments to be described. Since these media cannot be very accurately standardized, care was taken that the total quantity of either of them used in any one comparison of cultures was prepared in one lot. Petri dishes (10 cm) and Erlenmeyer flasks (200 cc) were used as containers, and the substrates were poured to the ordinary depth of 4 to 5 mm. In brief, no attempt was made to influence the growth of the cultures or to induce or inhibit variation by modification of media or culturing methods.

The term "mass isolate", as used in this paper, denotes that a culture originated from an unknown amount of any one or more of the several stages of pleomorphic *Ceratostomella ulmi*. It is applied to isolates from wood and insect specimens and is merely intended to differentiate the cultures, which may have started from several hyphae or spores, from monoconidium progenies.

For the isolation of single conidia, a micromanipulator of recent model and the Dickinson (*9, pp. 361–363*) technique were used. This method made it possible for the writer to be positive in each case that a culture originated from a single spore.

The system adopted for labeling the various cultures accumulated in this study is designed to indicate lineage at a glance. The original mass isolates are designated by numbers following a capital letter, as M669, A1202, W27, etc. All sector isolates are designated by numbers following a dash, and the monoconidium lines are designated by numbers in parentheses. For a "second generation" of monoconidium progenies, i. e., monosporous isolates from the first series of monoconidium lines, the parenthetical numbers are combined with small letters. For example, the sectors arising from mass isolate W27 are labeled W27–1, –2, –3, –4, etc.; monoconidium isolates from W27 are labeled W27 (1), (2), (3), (4), etc.; monoconidium isolates from sector types of this stock are labeled W27–1 (1), W27–1 (2), etc.; a second series of monoconidium isolates from previous monoconidium progenies are labeled either W27 (1a), etc. or W27–1 (1a), etc.; sector isolates from earlier sector isolates are labeled W27–2–1, etc.; sector isolates from monoconidium isolates are labeled either W27 (1–1), etc. or W27 (1a–1), etc.

OBSERVATIONS AND EXPERIMENTS

VARIATION IN MASS ISOLATES

The mass isolates of *Ceratostomella ulmi* obtained from trees of the New Jersey-New York-Connecticut area have been quite uniform, particularly with respect to their cultural characters on the media previously mentioned. A typical culture is shown in figure 1, *A*. The colony characteristics of the typical isolate (*15, 21*) may be briefly described as follows: Mycelium watery white, partly loosely aerial and waving, partly appressed, spreading; aerial mycelium pro-

nouncedly zonate in cultures exposed to fluctuations in light; aerial hyphae partly twisted and agglutinated together to form mycelial

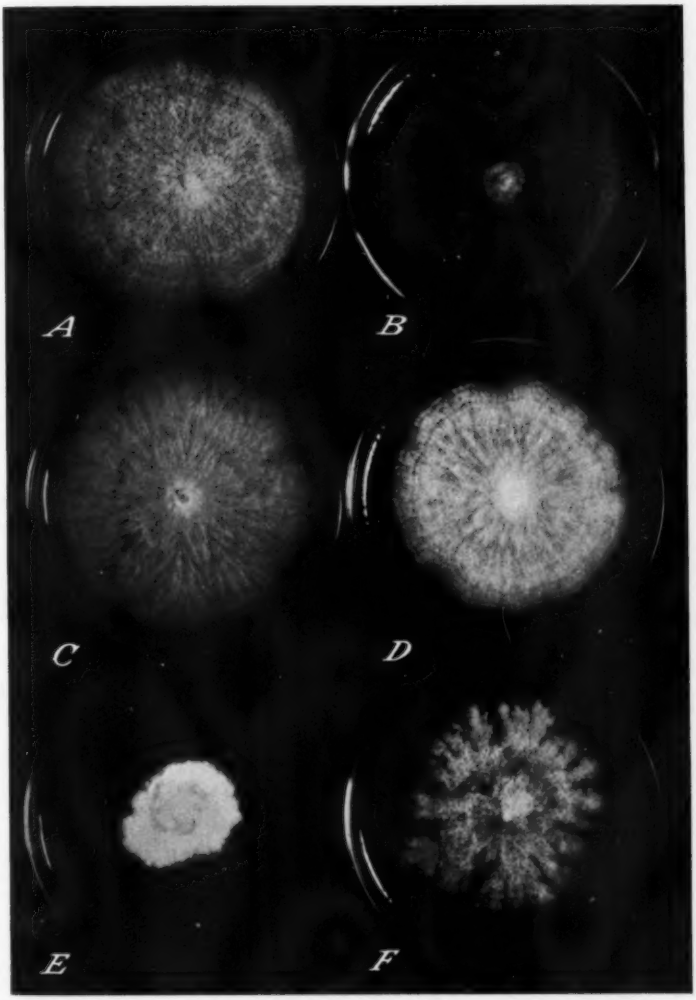

FIGURE 1.—Range of variation observed in mass isolates of *Ceratostomella ulmi* as shown by cultures on malt agar, incubated simultaneously at 23° to 25° C. in darkness for 4 days and then for 8 days at 17° to 20° on a laboratory table. ×½. *A*, A typical zonate isolate from a tree in New Jersey; *B*, a brown-colony type that produces numerous coremia on agar; *C*, a spreading, practically nonzonate, resupinate type; *D*, an isolate characterized by abundant aerial mycelium, intermediate growth rate, and pronounced zonation; *E*, a slowly spreading, decidedly aerial isolate originating from a tissue culture; *F*, the most yeastlike isolate recognized as *C. ulmi*.

strands, imparting to the aerial mycelium a fibrous appearance; colony becoming yeastlike-slimy and cream-colored progressively from the center as it ages.

In figure 1 are pictured six isolates chosen to represent the range of variation thus far recognized in mass isolates of *Ceratostomella ulmi*. Various types intergrading between the six shown in figure 1 have been noted, but, as suggested above, relatively few of the isolates have differed strikingly from the typical culture pictured in *A*. The remaining five cultures shown in figure 1 really represent the extremes of variation in cultural characters and may be briefly described as follows:

Figure 1, *B*, illustrates [4] a brown, coremium-prolific type that has been recognized only once, so far as the writer knows, in isolations made directly from field collections. In cultures incubated at 23° to 27° C., the brownish color did not become especially noticeable until the fifth day. In the routine diagnosis work of the laboratory those plates in which *C. ulmi* is recognized are discarded by the fourth d Thus it is quite possible that this brown-colony type of *C. ulmi* might have been noted more often had the system been different.

The brownish pigment is in the hyphae and appears to be of the same nature as that of the well-known blue-staining fungi of the genus *Ceratostomella*. It develops progressively from the center of the colony outward, but the hyphal tips of the colony margin remain dark hyaline, typical of fungi of this group.

The actual culture shown in figure 1, *B*, is of noteworthy origin. It was obtained by transferring primary conidia from the head of a coremium produced on an autoclaved elm chip to which ascospore slime from a perithecium had been transferred. The perithecium had formed from a combination, made by Swingle, of culturally typical isolates, one of which was a monoascospore progeny and the other a mass isolate from a diseased tree at Norfolk, Va. *C* illustrates a type of colony of closely appressed, practically, nonzonate, spreading habit. *D* is an isolate of intermediate growth rate, with a pronounced tendency to form abundant aerial growth in zones.

Figure 1, *E*, is a picture of the slowest growing mass isolate of aerial habit thus far identified as *Ceratostomella ulmi* by the author. In characteristics other than rate of colony spread it is similar to typical isolates of *C. ulmi*. This isolate has been repeatedly subcultured on the malt and potato-sucrose media and its growth has been consistently slow.

Figure 1, *F*, shows the most yeastlike culture thus far found to be *Ceratostomella ulmi*. The radial spread of the colony is relatively slow and the spreading hyphae are definitely more tortuous and more frequently branched than in the typical isolate. Conidia produced by budding accumulate rapidly.

The mass culture shown in figure 1, *F*, which has been studied under the label W22 (fig. 2, *A*) and will be mentioned frequently hereinafter, developed from spores transferred from a coremium that formed on a noncured, nonautoclaved cross section of wood.[5] This section of wood had been sprayed with a suspension of spores taken from coremia produced by typical isolate M669 (fig. 2, *C*). Weekly examinations of the wood section indicated that development of a first crop

[4] This culture was isolated from a diseased tree at Elmsford, N. Y., by workers in the Dutch Elm Disease Laboratory of Cornell University, located at the Boyce-Thompson Institute for Plant Research, Yonkers, N. Y. It is mentioned through courtesy of Dr. L. J. Tyler, instructor in plant pathology, Cornell University.

[5] Inoculation of wood section and reisolation by H. V. Wester, junior pathologist, Emergency Conservation Work.

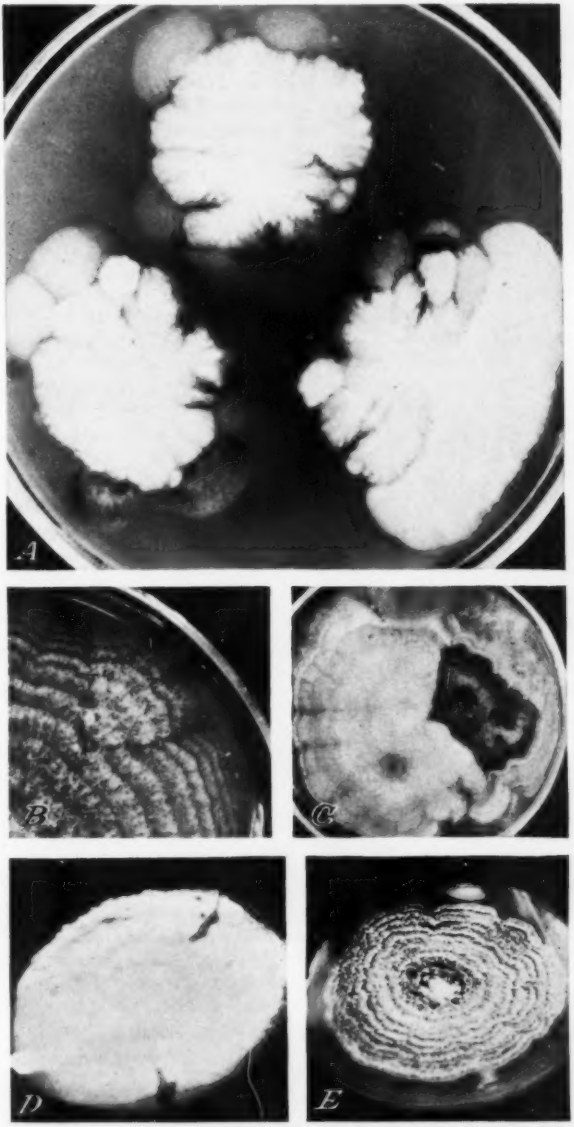

FIGURE 2.—Colonies showing sectors in mass isolates and a comparison between a parental and a sector type: *A*, A 20-day-old culture of W22 on malt agar, showing profuse sectoring characteristic of this yeastlike isolate. Transfers were made from 20 sectors in these three colonies, 14 of which were culturally different from the parental type and from one another. ×1. *B*, A section of a 12-day-old plate culture of A2102 on potato-sucrose agar, showing sector A2102–1. ×1. *C*, A culture of M669 approximately 15 weeks after isolation from a tissue culture, showing the brown sector M669–56. ×½. *D*, M669 9 to 10 months after original isolation from a tissue culture. Comparison of *C* and *D* shows change from typical to mycelial habit. ×¾. *E*, M669–56 9 to 10 months after original isolation but grown under same conditions as culture *C*, its parental type. The zoned ridges of *E* are made up of coremia with coremiospore slime running down the stalks. ×¾.

of coremia on it ceased 6 weeks after the suspension was applied; and a further 6 weeks elapsed before the coremium from which W22 was derived appeared. Prior to the appearance of the coremium which gave rise to W22, a number of other isolates considered to be typical *Ceratostomella ulmi* were used in further inoculations of wood sections in the same experiment, and these sections were incubated with the first. The wood sections were nontreated and were, of course, well populated by mites. Thus, though it is by no means necessarily the case, it is entirely possible that W22 is a variant from M669. Prior to the time that it was used as inoculum for the wood sections, M669 had produced no noteworthy variants but later observations showed it to be highly unstable.

W22 represented such a departure from the typical that the writer was not content to consider it *Ceratostomella ulmi* until (1) inoculation tests [6] showed that it produced symptoms characteristic of Dutch elm disease and (2) combinations [7] between it and minus strains produced perithecia of *C. ulmi*. W22 is of particular interest because it has been more prolific in sector production than any other isolate studied, though the difference may have been more apparent than real. It will be realized at once, upon consideration of their respective colony characteristics, that W22 was more suitable than the typical isolate for a study of sectoring.

As has been noted previously for this (*21*) and other fungi, the stock cultures of *Ceratostomella ulmi* have tended to become more and more mycelial with repeated transferring. Production of secondary conidia has been reduced (though no mass isolate kept in stock by the writer has completely discontinued the production of secondary conidia), while the proportion of aerial mycelium has increased. This change may be illustrated by a comparison of *C* and *D* of figure 2. *C* shows the culture M669 after it had been in stock for about 15 weeks; *D*, after about 10 months. The writer considers that this trend to mycelial habit may perhaps be the result of the development of unnoticed variants capable of overrunning the parental colony and becoming dominant in transplants because of their spreading mycelium

SECTORING OF MASS ISOLATES

Sharply defined sectors in the margin of the growing colony and decidedly different patches or tufts on the colony surface have been noted in a number of mass cultures or isolates of *Ceratostomella ulmi*. However, for the sake of assurance in making transfers, only peripheral sectors have been isolated for further study and comparison. Figure 2, *B*, is an enlarged reproduction of one-fourth of a colony of typical isolate A2102 showing a very well-defined peripheral sector.

Sectoring of *Ceratostomella ulmi* was first noted in isolate W27, in December 1933. The isolate had been in stock about 2 months at that time and had been subcultured frequently. The original isolate was quite typical *Ceratostomella ulmi*. The first sectors noted had made an especially bizarre growth, being more aerial and fluffy, yet had spread more slowly than the parent colony. No secondary conidia were produced on the hyphae of these odd sectors, and as the cultures aged, numerous sterile stalks, similar in structure to coremial stalks,

[6] Inoculations and reisolations by S. J. Smucker, junior pathologist, Emergency Conservation Work.
[7] Combinations for perithecium formation by R. U. Swingle, agent, Division of Forest Pathology.

were formed. For the most part the latter were hyaline or white, but patches of them were bright yellow or dull yellow to grayish black. A comparison of the above-described variant [8] with the parental type and two sector types commonly noted later in W27 is presented in figure 3, *A*, *B*, *C*, and *D*. The original isolate and the three aberrant forms are representative, respectively, of the usual mass isolates and of the most frequently arising distinct sector types that have appeared

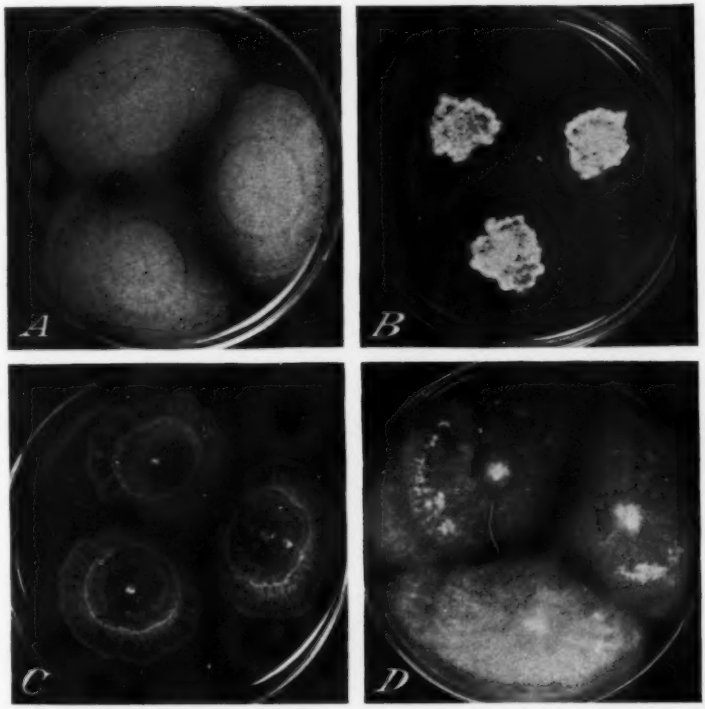

FIGURE 3.—Aberrant progenies as compared with their parental stocks: *A*, W27, a typical isolate (after 17 months in culture) that gave rise to a number of aberrant forms. Compare with *B*, *C*, and *D*. All four cultures grown on malt agar at room temperature for 6 days. *B*, W27–1, a frequently appearing variant type which grew slowly, did not sporulate, and produced numerous stalks. *C*, W27–2, another frequently appearing variant type which is slower spreading than its parental type and which develops a brown color and numerous coremia within 4 to 7 days after transfer. *D*, W27–3, a third variant type which appeared frequently. It is faster spreading and more resupinate than the parental type. All ×½.

in the mass isolates to which particular attention has been given in this study. The variant type W27–2 was very much like culture S65, shown in figure 1, *B*, but the photograph of the latter was taken when the culture was older. Culture W27 (fig. 3, *A*), had been in stock for 17 months at the time it was photographed, and was definitely more mycelial than it was when first isolated.

The second mass isolate (M669) that was accorded special study is pictured in figure 2, *C*, which shows a very striking sector that

[8] S. J. Smucker found a sector type practically identical with W27–1 in a typical isolate of *Ceratostomella ulmi* in February 1934. (Unpublished notes of the Division of Forest Pathology.)

appeared in it. Figure 2, *D* and *E*, represents a comparison of this variant, M669–56, with its parental stock after both had been repeatedly subcultured for 9 to 10 months. Attention has been called to the shift of the parental stock to a more mycelial habit. Prior to this change the parental type M669 gave rise to a number of striking variants, but their study was discontinued because the stock culture of the parental type could no longer be considered satisfactory for comparison with them.

The brown variant M669–56 is very much like the brown culture S65 (fig. 1, *B*) and also closely resembles W27–2 (fig. 3, *C*). It is worthy of special note that in a preliminary test M669–56 proved fully as pathogenic, if not more so, than subcultures of its parental type. However, the inoculations for variant and parental subculture involved only 10 trees each, of a uniform lot of 5-foot greenhouse elms. The fact that the parental stock, M669, had become definitely mycelial before the inoculations were made is a further reason for reserve in the evaluation of the results of this preliminary comparison of pathogenicities.

The origin of isolate W22 has been explained previously and mention has been made of its prolific production of variants. The pronounced tendency of W22 to sector is illustrated in figure 2, *A*. From the three colonies in this one Petri dish, 20 separate sector transfers were made, but some of the group were later considered duplicates. However, 14 of the 20 could readily be distinguished from the parental type and from one another. Even though it was repeatedly subcultured, W22 continued to produce distinct sectors and many of its variant types themselves sectored profusely. There has been no end to the appearance of sectors from this stock.

A representative group of sector isolates from W22 is shown in figure 4. These cultures, on potato-sucrose agar, were prepared in one lot and incubated simultaneously for 4 days at 27° C. and then moved to a laboratory table. The photographs were taken on the ninth day from the time transplants were made. Brief notes, made at the time of photographing and giving only the most striking characteristics, are listed below for each of the types shown:

A, W22–27.—Could hardly be differentiated from typical *Ceratostomella ulmi* isolate.

B, W22–28.—Much like typical *C. ulmi* isolate. Lacking zonation.

C, W22–24–5.—Heavily sporulating and spreading. Conidia slightly smaller than those of typical *C. ulmi*.

D, W22–2.—Hardly distinguishable from typical isolate except by a slower growth rate and irregular colony margin.

E, W22–22–3.—Profuse aerial conidiophore formation and sporulation. Gross appearance much like that of typical *C. ulmi* isolates except for smaller diameter of colonies.

F, W22–17.—Hyphae delicate. Heavily sporulating.

G, W22–4.—Resupinate and heavily sporulating. Colony lemon yellow with black center.

H, W22–21.—Colony margin feltlike with many nonfruiting hyphal branches suggestive of conidiophores. Very small conidia borne within half radius of the colonies.

I, W22–22.—Spreading habit with considerable nonfruiting aerial mycelium. Sporulation light.

J, W22–22–1.—Dense aerial growth in which the tendency to form strands is less marked than in typical *C. ulmi*. Zonation barely perceptible.

K, W22–6.—Conidiophores of greater average length than in typical cultures of *C. ulmi* and conidia smaller because they are slenderer, though not shorter, than in typical *C. ulmi*. Hyphae more delicate than the average.

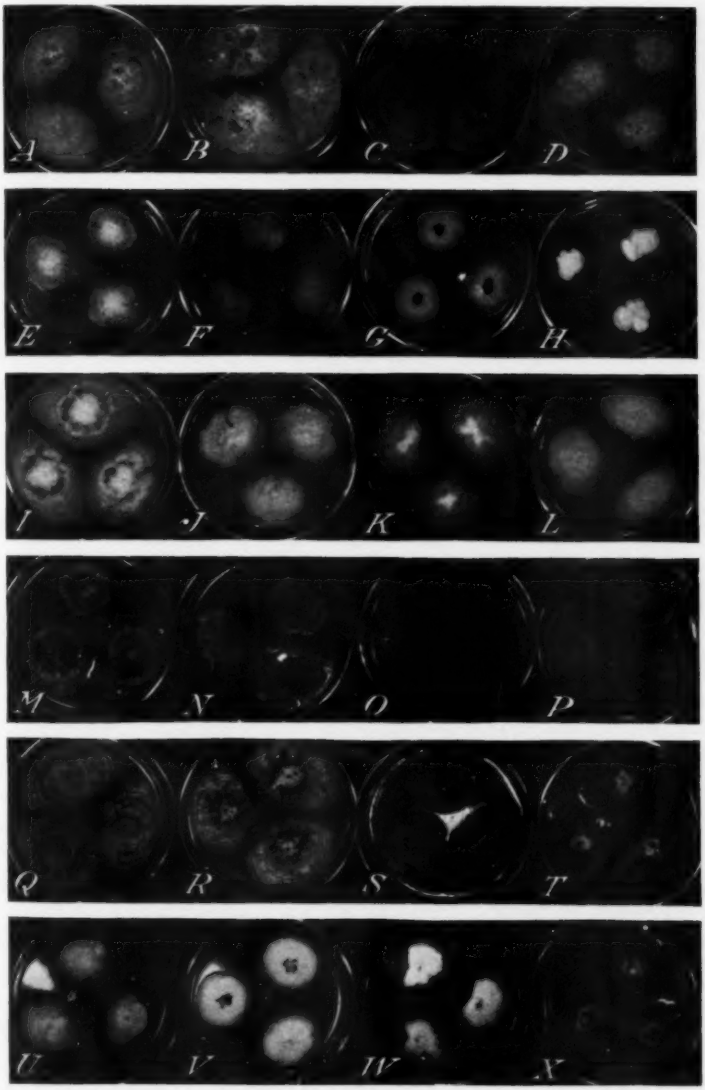

FIGURE 4.—Range of sector types isolated from W22: 9-day-old cultures on potato-sucrose agar prepared in one lot, incubated simultaneously for 4 days at 27° C., and then moved to a laboratory table. An attempt has been made to group, in horizontal rows, types of general resemblance: *A*, W22-27; *B*, W22-28; *C*, W22-24-5; *D*, W22-2; *E*, W22-22-3; *F*, W22-17; *G*, W22-4; *H*, W22-21; *I*, W22-22; *J*, W22-22-1; *K*, W22-6; *L*, W22-16; *M*, W22-22-5; *N*, W22-30-2; *O*, W22-7; *P*, W22-7-1; *Q*, W22-11-1; *R*, W22-30-1; *S*, W22-9; *T*, W22-10; *U*, W22-23; *V*, W22-20; *W*, W22-3; *X*, W22-3-1. ×½

L, W22–16.—Lacking the tendency to "strand" formation typical of *C. ulmi*. Dense aerial growth slow to form cephalosporic heads of spores.

M, W22–22–5.—Much of mycelial mat is beneath surface of agar. Pronounced tendency to "strand" formation. Hyphae contain an olivaceous-brown pigment which appears to be quite uniformly distributed throughout colony. Coremia numerous.

N, W22–30–2.—Conidia less than average size for typical *C. ulmi* and more definitely clumped in cephalosporic fashion. Hyphae brown-colored in the spots from which coremial stalks arise.

O, W22–7.—Spore heads on conidiophores nearer colony margin than in any other type shown. Conidia more nearly spherical than in typical *C. ulmi*. Coremia numerous. Dark pigment more intense than in others of group but apparently of the same nature.

P, W22–7–1.—Spreading, but with conidia like those of *O*. Coremia fewer but much larger than those of *M*, *N*, and *O*.

Q, W22–11–1.—Strands numerous and lengthy. Brown pigment similar to that in *M* but more diffuse, giving a pale-olivaceous color.

R, W22–30–1.—Sporulation very slight near margin but heavy at half radius. Center of colony of matted mycelium surrounded by a zone with olivaceous pigment. Very well defined zonation.

S, W22–9.—Pronounced tendency to formation of mycelial strands. No conidia near colony margin, but toward the center of the colony sporulation is heavy. Conidia of less than average size. Pigment similar to and more abundant than that in *M*.

T, W22–10.—Much like *S*, but sporulating nearer the colony margin and with more aerial mycelium.

U, W22–23.—Nonsporulating, and with downy aerial mycelium showing fairly well defined zones. Stalklike bristles in colony centers are white to yellowish.

V, W22–20.—Nonsporulating. Much like *U* but with a striking tendency to form jet-black stalks in the center of the colony, and lacking zonation.

W, W22–3.—Nonsporulating. Much like *U* and *V* but producing sterile yellowish stalks more abundantly. Colonies markedly segmented.

X, W22–3–1.—No sporulation. Spreading, yet definitely zonate. Gross appearance much like that of *T* except that this type has numerous, erect, nonbranched hyphae of brownish color.

Many of these variant or sector types from W22 were of such appearance that they might not have been recognized as *Ceratostomella ulmi* had they been of unknown origin. Several have thus far failed to produce secondary conidia; some have failed to produce coremia though developing very numerous secondary conidia; and some have produced either coremia or secondary conidia or both more abundantly than typical isolates of *C. ulmi*. Though the original stock could not by any means be considered a typical isolate of *C. ulmi*, it has produced a number of sector types that are hardly distinguishable from a typical isolate.

It will be noted (fig. 4) that *E*, *J*, and *M*, are sector isolates from *I* and that *P* and *X* are sector isolates from *O* and *W*, respectively. The types shown in *M*, *N*, *O*, and *P* are much more inclined to coremial formation than are the others, and types shown in *U*, *V*, *W*, and *X* are nonsporulating lines. The culture pictured in *W* is typically white, slow spreading, and nonzonate. The similarity of the dark segments of the colonies shown in *W* suggests that the material transplanted to this plate was of two types. The dark type was not apparent in the test-tube stock culture from which the transplants were made. This instance clearly illustrates a difficulty encountered in this study, namely, that of maintaining reliable stock cultures of some of the frequently sectoring types.

SECTORING OF MONOCONIDIUM PROGENIES

It was essential to determine whether the sectoring noted in mass cultures might be mere separation of different components of hetero-

geneous isolates of *Ceratostomella ulmi*, or, of greater importance, whether it might represent the sudden origin of entirely different cultural races of the fungus. Accordingly, monoconidium progenies were started from three of the mass isolates previously mentioned. Monoconidium lines were also secured from a number of the sector types from W22 to determine whether their striking and distinguishing features would be continued and whether the single-spore progenies would sector as had their parental stocks.

Sectors appeared in the single-spore progenies from all three stocks, but more frequently from the W22 lines than from progenies of either of the other mass isolates. Figure 5, *A–H*, shows two monoconidium

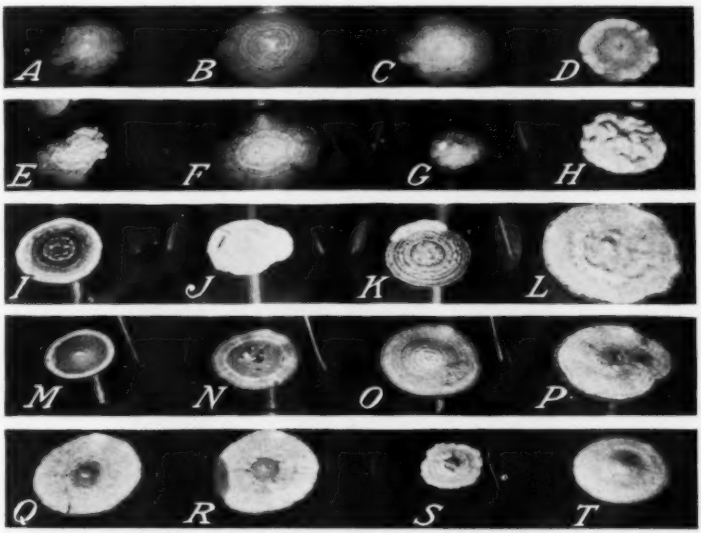

FIGURE 5.—Variant production by and differences between monoconidium isolates from W22 and certain of its early variants. The cultures are on malt agar in Erlenmeyer flasks. Photographs were taken after incubation for 12 days at room temperature: *A*, Monoconidium progeny W22 (38). *B*, *C*, and *D*, three variants from *A*: *B*, W22 (38–1); this culture could not be differentiated from the typical *Ceratostomella ulmi* isolate; note the well-defined sector. *C*, W22 (38–2, *D*, W22 (38–3). *E*, monoconidium progeny W22 (53). *F*, *G*, and *H*, three variants from *E*: *F*, W22 (53–1); *G*, W22 (53–2); note the "patch" variant on the surface; *H*, W22 (53–3). *I*, *J*, *K*, *L*, *M*, *N*, *O*, and *P*, eight monoconidium progenies from mass-sector isolates from W22: *I*, W22–4 (1); *J*, W22–5 (1); *K*, W22–7 (1); *L*, W22–11 (1); *M*, W22–17 (1); *N*, W22–18 (1); *O*, W22–22 (1); *P*, W22–30 (1). Note the distinct sectors in *K*, *O*, and *P*. *Q*, *R*, and *S*, three monoconidium isolates from W22–10: *Q*, W22–10 (1); *R*, W22–10 (2); *S*, W22–10 (3). *T*, W22–10 (3–1), a variant from W22–10 (3). ×½.

isolates from the original W22 stock in comparison with three variants from each of them. A very well defined sector is shown in colony W22 (38–1), and a very noticeable "patch" variant is shown on the surface of colony W22 (53–2).

Sectors likewise appeared in single-spore progenies started from the early sector isolates from W22. Three of the eight colonies shown in figure 5, *I–P*, namely, W22–7 (1), W22–22 (1), and W22–30 (1), have very distinct sectors. These eight cultures are of interest because of the range of variation they represent as well as because of the sectors shown.

Strikingly different types were sometimes obtained by starting only a few single-spore progenies from a mass-sector isolate (fig. 5, *Q*, *R*, and *S*). As will be seen upon examination of the latter illustrations, W22–10 (1) and W22–10 (2) are very much alike, but the third single-spore progeny from the same mass, W22–10 (3), is decidedly different

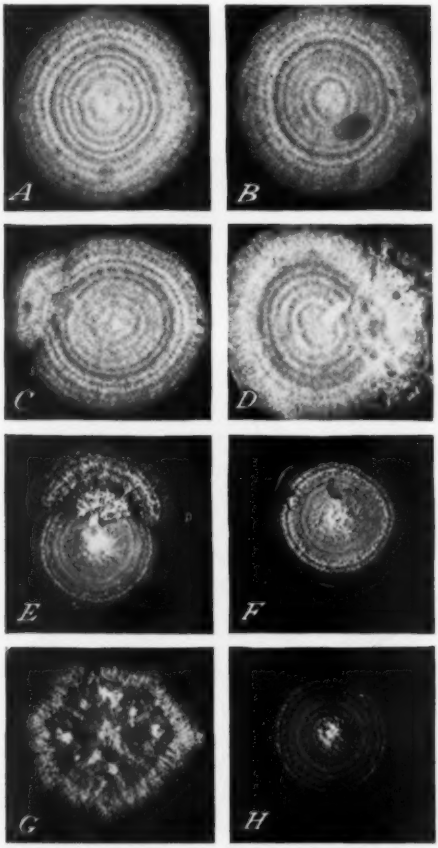

FIGURE 6.—Variations in "second generation" monoconidium progenies, cultures on potato-sucrose agar incubated for 6 days at 27° C. *A*, Monoconidium isolate W27 (5). Compare with *B*, *C*, and *D*, which are three monoconidium progenies started from *A*. *B*, *C*, and *D*, W27 (5*a*), W27 (5*b*), and W27 (5*c*), respectively. Note that each of the latter has a more or less distinct sector. *B*, Monoconidium isolate W22 (48). Compare with *F*, *G*, and *H*, which are three Monoconidium progenies started from *E*. *F*, *G*, and *H*, W22 (48*a*), W22 (48*b*), and W22 (48*c*), respectively. Note the striking difference between *G* and its sister progenies *F* and *H*. The spores from which *F*, *G*, and *H* were started were all taken from a single transfer from type *E*. ×⅓.

from them. Yet culture W22–10 (3) soon produced a sector type, W22–10 (3–1) (fig. 5, *T*), which is much like W22–10 (1) and W22–10 (2), although it is readily differentiated from them.

Seven of the sectoring monoconidium progenies were chosen for the second isolation of single conidia, but only three progenies started from each of them were studied. For example, monoconidium culture

W27 (5) was used as source material for three further single-spore progenies, W27 (5*a*), (5*b*), and (5*c*). This experiment was undertaken with the twofold purpose of determining whether (1) the production of sector variants would continue and whether (2) the few spores selected would give cultures of different types, as had happened in W22–10.

These second-generation monoconidium isolates produced well-defined sectors without delay (fig. 6, *B*, *C*, and *D*). Although no attempt was made to isolate and record every sector variant, it may be stated that the frequency of sectoring by these second-generation monoconidium progenies was not appreciably less than that of the first or primary progenies. It will be noted in the examination of figure 6 that distinct sectors are present in cultures *B*, *C*, and *D*, which are, respectively, W27 (5*a*), W27 (5*b*), and W27 (5*c*). The primary monoconidium line from which *B*, *C*, and *D* was started is shown for comparison in figure 6, *A*.

Even though three second-generation single-spore progenies from any individual primary single-cell progeny were studied, in two cases out of seven decidedly different lines were obtained. Referring to figure 6 again, it is obvious that culture *G* was quite unlike cultures *F* and *H*, but all three grew from cells taken from an apparently homogeneous test-tube stock of culture *E*, which was monoconidium isolate W22 (48). As shown by the striking sector in the colony pictured, W22 (48) was unstable.

Such results indicate a high degree of instability in the genotypic constitution of conidia of *Ceratostomella ulmi*, which are direct descendants, many cells removed, of course, from a single spore. In other words, they are evidence of fortuitously unequal cell divisions in the asexual reproduction of the fungus. Supporting cytological evidence on this point cannot be given; and, because of the small size of the nuclei of hyphae and conidia of *C. ulmi*, it seems unlikely that such evidence will be forthcoming in the near future. Cytological study of *C. ulmi* has, to be sure, been rather limited thus far, but it appears safe to consider that its conidia are quite regularly single-celled (*21*) and that the hyphal cells and conidia are typically uninucleate.

DISCUSSION

From the work of Swingle (*19*) and that described in the preceding pages it must be concluded that the species *Ceratostomella ulmi* comprises a wide range of cultural races, some of which are highly variable. This view by no means precludes the presence within the species of relatively stable lines. Because (1) *C. ulmi* has become known to mycologists only recently, (2) it comprises a wide range of cultural races, (3) single-spore progenies of it have given rise to such striking variants, and (4) geneticists are agreed that wide crossing or hybridization commonly gives highly variable or unstable progenies, the writer is led to suggest that this pathogenic species, new in a group that has long been known as largely saprophytic, may have arisen by the union of lines of different nonpathogenic or weakly pathogenic species of *Ceratostomella* or *Graphium*.

The phenomenon of sectoring in culture has been recorded for many fungi and has been referred to by different authors (*1*, *2*, *3*, *6*, *8*, *14*, *17*) as chimera formation, saltation, mutation, dissociation, and

variation. Certain workers (*1, 2, 8, 11, 14, 17*) have striven to determine the true nature and to interpret the biological significance of this common phenomenon. Apparently because of the difficulties encountered in cytological study of fungi, there is not yet general agreement concerning either the mechanics or the significance of the changes involved. This is evidenced by the common use, for the sectoring phenomenon, of the above list of terms, of which at least two (mutation and dissociation) signify widely different interpretations. The problem is greatly complicated by the differences in the cytological features of the different groups of fungi.

Although the origin of sector variants in cultures of fungi is not yet fully understood, considerable progress has been made in recent years. For example, Dickinson (*8*), finding sectors in *Fusarium* cultures which he knew to be the progeny of a single nucleus, was able to conclude definitely that the changes involved the nucleus and were thus worthy of the name "mutations." The writer is inclined to the opinion that sector variants of *Ceratostomella ulmi* described in this paper are true mutants.

With respect to the aberrant cultural races obtained as mass isolates from field specimens, there is, of course, a possible origin other than mutation, namely, sexual reproduction. Buisman (*5*) has recorded the occurrence of perithecia of *Ceratostomella ulmi* in nature in Europe, and Swingle (*19*) has shown that complementary plus and minus strains of the fungus have been present in close proximity in the United States.

The observations on monoconidium progenies described in this paper are considered conclusive evidence that strikingly different cultural races of *Ceratostomella ulmi* arise fortuitously; and the facts at hand concerning the range of variation shown by the mass isolates from field specimens suggest that variants of this important pathogen are arising in nature. The importance of this information in the diagnosis of specimens from suspected cases of Dutch elm disease is apparent. It may be of far greater importance to those concerned with the study of the disease, in view of the fact that careful work with monosporous cultures of other fungi (*7, 12, 13, 18*) has conclusively demonstrated that variant types, originating in the same manner as these sector variants of *C. ulmi*, differ from their parental types in pathogenic capabilities.

SUMMARY

The observations and experiments recorded in this paper show that the species *Ceratostomella ulmi* comprises a wide range of cultural races.

Strikingly different cultural races appeared as sectors in isolates from diseased trees and in monoconidium progenies from these isolates.

The importance of these facts to those concerned with the cultural diagnosis of cases of Dutch elm disease is indicated.

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THE TIME FACTOR IN POLLEN-TUBE GROWTH AND FERTILIZATION IN BARLEY[1]

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INTRODUCTION

The statement is commonly made that fertilization of the ovule in the cereal crops occurs within 24 hours after pollination. More definite estimates are rare. In studying material collected during the winter of 1934–35, stages in the pollination and fertilization of

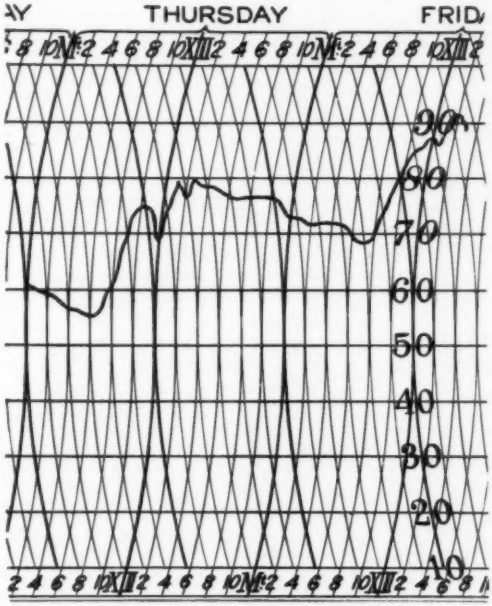


FIGURE 1.—Thermograph record for Thursday and Friday, February 28 and March 1, 1935.

barley were found showing that a surprisingly short time may elapse between the act of pollination and the beginning of growth in the young seed.

MATERIAL AND METHODS

A number of hand pollinations were made in the greenhouse at the Arlington Experiment Farm, Arlington, Va., near Washington, D. C., by the dusting method,[2] on the Hannchen variety of *Hordeum distichon*

[1] Received for publication Oct. 8, 1936; issued May 1937.

[2] POPE, M. N. A RAPID METHOD FOR MAKING SMALL GRAIN HYBRIDS. (Note). Jour. Amer. Soc. Agron. 25: 771–772. 1933.

palmella (C. I.[3] no. 531), at 9:30 a. m., February 28, 1935. Figure 1 shows the thermograph record from pollination to the time the last of the samples here described was killed and fixed. Pollinated ovaries were removed from lemma and palet and immediately killed and fixed in Wilson's modification of Bouin's fluid at intervals of 5 minutes for the first hour and hourly thereafter to the age of 3 days. Frontal and sagittal sections were cut 15μ thick and stained with Flemming's triple and iron-alum haematoxylin, the latter being the more satisfactory.

OBSERVATIONS

When the barley flower is ready for pollination the two plumose stigmas of the ovary are spread wide and a median sagittal section shows the matured female gametophyte. Between the pair of synergids at the proximal tip of the embryo sac lies the naked egg surrounded by cytoplasmic protoplasm, which encloses the two polar nuclei and extends in wavy strands into the sac cavity, reaching the mass of approximately 35 matured antipodals at the chalazal side of the sac. The pollen grain (pl. 1, *A*) has already produced a tube nucleus roughly spherical in shape and two oval, more or less elongated, male gametes lying in the cytoplasm.

Under the conditions as described, the pollen germinated within 5 minutes after falling on a stigma hair (pl. 1, *B*) and the male gametes entered the pollen tube within 10 minutes (pl. 1, *C*). The course of the pollen tube from its position at germination down the stigma and ovary was not traced in this study. Lermer and Holzner[4] believe that the pollen tube follows the S-shaped band of elongated thin-walled cells extending from the stigma branch inward and downward to the axis of the ovary, where the band joins the one from the other stigma branch to enter the cone-shaped upper end of the ovary cavity. From this point the tube traverses the outer integument to the micropyle. However, Krauss[5] found a pollen tube in ovary wall tissue other than that described by Lermer and Holzner and another proceeding downward between the outer integument and the inner epidermis of the ovary. Plate 1, *D*, shows the swollen tip of a pollen tube which was evidently just emerging from between these tissues and was at the level of the egg in an ovary 45 minutes after pollination.

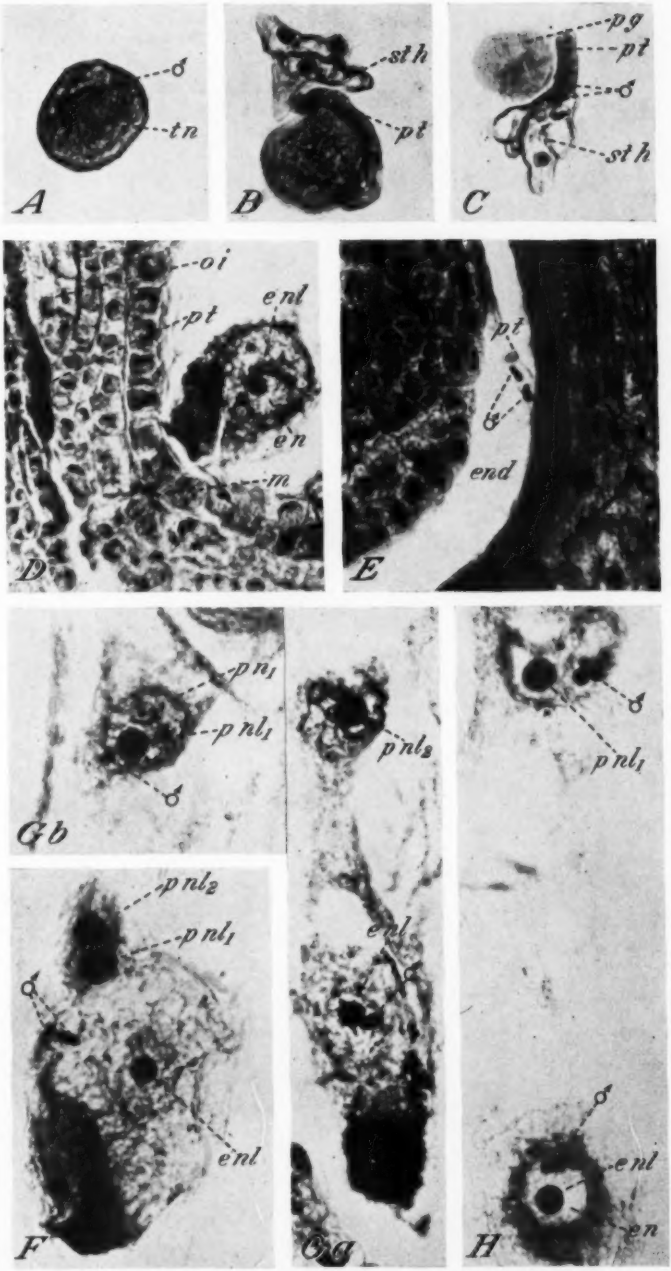
[3] C. I. denotes accession number of the Division of Cereal Crops and Diseases (formerly Office of Cereal Investigations).

[4] LERMER, J. K., and HOLZNER, G. BEITRÄGE ZUR KENNTNIS DER GERSTE. 106 pp., illus. Munich. 1888. (See p. 4.)

[5] KRAUSS, L. ENTWICKLUNGSGESCHICHTE DER FRÜCHTE VON HORDEUM, TRITICUM, BROMUS UND POA MIT BESONDERER BERÜCKSICHTIGUNG IHRER SAMENSCHALEN. Jahrb. Wiss. Bot. 77: [733]–808. illus. 1933. (See p. 743.)

EXPLANATORY LEGEND FOR PLATE 1

A, Ungerminated pollen grain showing male gametes and tube nucleus. × 600.
B, Germinated pollen grain 5 minutes after pollination. × 600.
C, Male gametes in a section of the pollen tube 10 minutes after pollination. × 600.
D, Enlarged tip of pollen tube just below edge of outer integument and at level of egg 45 minutes after pollination. × 600.
E, Male gametes in a section of the pollen tube in the endostomium near the micropyle. × 600.
F, Male gametes in the cytoplasm surrounding the egg 61 minutes after pollination. × 1,075.
G, Egg and polar nuclei of an ovule 45 minutes after pollination. *a*, Egg with male gamete in contact. Also one of the polar nuclei. × 1,075. *b*, The other polar nucleus with male gamete in contact. × 1,075.
H, Egg and polar nucleus 2 hours after pollination. Male gamete at egg has become vacuolated. Male gamete at polar nucleus still localized but diffusing and showing several nucleoli. × 1,075.
δ, Male gamete; *tn*, tube nucleus; *sth*, stigma hair; *pt*, pollen tube; *pg*, pollen grain; pn_1, pn_2, polar nuclei; *oi*, outer integument; *enl*, egg nucleolus; *en*, egg nucleus; *m*, micropyle; *end*, endostomium; pnl_1, pnl_2, polar nucleoli.



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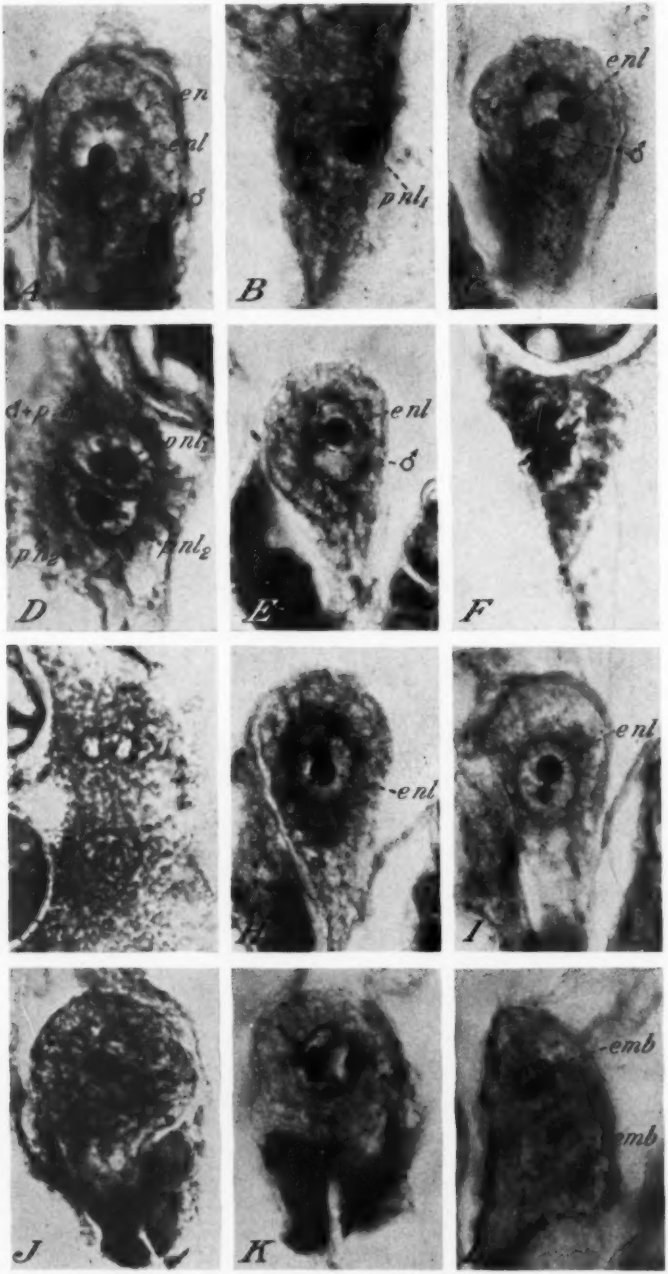

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In another ovary, 40 minutes after pollination (pl. 1, *E*), the two male gametes were in a section of the pollen tube lying in the endostomium near the micropyle. In a still later stage, 35 minutes after pollination, the male gametes had been freed from the pollen tube into the egg sac but had not yet reached the female nuclei. Plate 1, *F*, shows a similar stage in material killed and fixed 61 minutes after pollination.

In two out of seven 45-minute ovaries sectioned, the swollen male gametes are seen to have "arrived" (pl. 1, *G*, *a*, *b*) and to be apparently within the membranes investing the egg nucleus and one of the polar nuclei.

Two hours after pollination (pl. 1, *H*) the male gamete in contact with the egg had become vacuolated and less regular in outline, and the one in contact with one of the polar nuclei had lost its former shape and appeared as a much larger mass containing a number of spherical nucleoli.

In the 4-hour stage the male gamete in the egg (pl. 2, *A*) had enlarged, becoming an oval mass considerably larger and staining less deeply than at 2 hours. It may contain two or more small nucleoli. The polar nuclei at this stage (pl. 2, *B*) had visible boundaries and their large nucleoli maintained their sharp outlines, but there were generally present in the nucleoplasm of both varying numbers of small and medium-sized nucleoli presumably originating from the male gamete or arising from its stimulating effect.

By 5 hours after pollination the male gamete in the egg (pl. 2, *C*) had begun to take on the appearance of a spherical sector of the nucleus. The egg nucleolus still had a sharp outline. The nucleoli of the polar nuclei (pl. 2, *D*), on the other hand, were no longer perfectly spherical, and the chromatin network had become heavy and well distributed throughout both of them, indicating that both nuclei had become active. Additional small or medium-sized nucleoli were generally present in both, and the male gamete as a discrete body had disappeared.

At 6 hours the rather sharp sectorlike appearance of the male gamete in the egg had given place to a loose, somewhat heavy network still occupying but a portion of the nuclear mass and extending from the nuclear membrane inward toward the nucleolus (pl. 2, *E*). Union of the other male gamete with the two polar nuclei had occurred and division figures or daughter endosperm cells had developed. Of five ovules sectioned, one showed the late prophase (pl. 2, *F*), one was in telophase (pl. 2, *G*), and in the remaining three the first division had been completed.

EXPLANATORY LEGEND FOR PLATE 2

A, Egg 4 hours after pollination. Male gamete has enlarged greatly. Outline of egg nucleolus sharply defined.
B, Polar nucleus 4 hours after pollination. Male gamete has diffused. Outline of female nucleolus sharply defined.
C, Egg 5 hours after pollination. Male gamete a conical sector of the nucleus.
D, Polar nuclei 5 hours after pollination. Male gamete well diffused through one of them. Both female nucleoli losing their sharp outlines.
E, Egg 6 hours after pollination. Male gamete becoming diffuse.
F, Fusion nucleus 6 hours after pollination; in prophase.
G, Fusion nucleus 6 hours after pollination; in late telophase.
H, Egg 7 hours after pollination.
I, Egg 11 hours after pollination. Fertilization probably complete.
J, Egg 13 hours after pollination. Beginning prophase.
K, Egg 14 hours after pollination. Prophase.
L, Egg 15 hours after pollination. Two-celled embryo.
All × 1,075. *en*, Egg nucleus; *enl*, egg nucleolus; ♂, male gamete; pnl_1, pnl_2, polar nucleoli; pn_1, pn_2, polar nuclei; *emb*, embryo cell.

After this division the naked daughter endosperm cells migrated along the protoplasm strands laterally and divided about 9 to 10 hours after pollination to form the four-celled endosperm stage, and again, at about 13 to 14 hours after pollination, to give a coenocytic endosperm, the eight cells of which were located at intervals along the protoplasmic strands in the cytoplasm of the nucellus. During this time the male gamete in the egg cell became more and more diffuse within the female nucleoplasm (pl. 2, *H*), until the egg at 11 hours (pl. 2, *I*) contained one female nucleolus of egg origin and one or more smaller nucleoli of probable male origin in a mass of rather evenly distributed reticulum. At 13 hours the reticulum had begun to thicken and shorten to produce a prophase (pl. 2, *J*), which is still further developed at 14 hours (pl. 2, *K*). Fifteen hours after pollination ovules commonly showed eight naked widely separated endosperm cells. At this time the fertilized egg in one ovule appears in late prophase, and two show telophases or two-celled embryos (pl. 2, *L*).

A summary of all stages described is given in table 1.

TABLE 1.—*Stages in pollen-tube growth and fertilization in barley*

Time elapsed after pollination	Pollen	Egg	Fusion nucleus
5 minutes	Germinated		
10 minutes	Male gametes in pollen tube.		
45 minutes	End of pollen tube at edge of outer integument (1).[1]	Male gamete in contact (2).[1]	Male gamete in contact (2).[1] Polar nuclei migrating toward antipodals.
2 hours		Male gamete larger and vacuolated.	Takes position proximal to antipodals. Male gamete diffused. Larger bulk and small to medium nucleoli.
4 hours		Male gamete an enlarged oval mass containing 2 or more nucleoli within female nucleus.	Polar nuclei still not fused.
5 hours		Male gamete a spherical sector of the egg nucleus. Outline of egg nucleolus sharp.	Outline of female nucleoli rough. Smaller nucleoli present in both polar nuclei. Male gamete not well defined.
6 hours		Male sector of egg nucleus became progressively more diffuse.	Prophase (1);[1] telophase (1);[1] 2 endosperm cells (3).[1]
10 hours			Second division of endosperm occurring.
11 hours		Reticulum evenly distributed and contained 1 large and 1 or more smaller nucleoli.	3 to 5 endosperm cells.
13 hours		Beginning prophase	
14 hours		Late prophase (1);[1] metaphase (2).[1]	
15 hours		Prophase (2);[1] late telophase (2);[1] metaphase (1).[1]	8 endosperm cells.

[1] Figures in parentheses indicate number of observations.

SUMMARY

Pollen germinated within 5 minutes after reaching the stigma in *Hordeum distichon palmella*, variety Hannchen, under greenhouse conditions as described. The male gametes had entered the pollen tube within 10 minutes and had reached the level of the micropyle in 40 minutes. Within 45 minutes the two male gametes had entered the egg sac through the micropyle and one had reached or entered the egg nucleus, and the other, one of the polar nuclei. The fusion nucleus produced division figures and daughter endosperm cells within 6 hours after pollination. Division figures were present in the fertilized egg at the 14-hour stage, at which time there were in the ovule six to eight naked, rapidly migrating endosperm cells. The first division of the fertilized egg was completed within 15 hours after pollination.

CONSTRUCTION OF YIELD TABLES FOR NONNORMAL LOBLOLLY PINE STANDS[1]

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INTRODUCTION

Normal-yield tables are generally used to predict growth and yield from pure, fully stocked, even-aged, second-growth stands. Although valuable as standards, such tables can be applied directly only to occasional stands. Indirect application by the use of converting factors gives only approximate estimates for the large majority of stands, which are either not normally stocked or are made up of more than one species, or both.

The purpose of this paper is to describe a method to be used in the construction of loblolly pine yield tables that can be directly applied to even-aged, second-growth stands, either pure or mixed and of different densities of stocking. This method, based on an application of a growth curve, tends to minimize the errors due to personal bias in freehand curve fitting; it permits the use of mechanically selected rather than ocularly selected plots; and it measures the applicability of the tables to data similarly collected.

THE DATA

Data were taken on 150 mechanically selected, temporary sample plots during the course of a study of the loblolly pine pulpwood resources of the Coastal Plain and eastern Piedmont sections of Virginia, North Carolina, and South Carolina. One plot per 30 square miles was examined in those counties in which there were commercial stands of loblolly pine (*Pinus taeda* L.). The general procedure of the field parties was to travel along roads previously marked on maps of the territory, stopping at 5-mile intervals to establish and examine a plot. The plots were located by measuring 5 chains on an offset perpendicular to the road and to the right of the direction of travel, the 5-chain point being taken as the northeast corner of the plot. If the selected spot fell on agricultural land, the nearest forest within one-fourth mile was visited and a 3-chain offset made perpendicular to its edge. Stands were deemed satisfactory for the establishment of yield plots if they fulfilled the following specifications: (1) At least 10 percent of the number of trees in the main crown story should be loblolly pine; and (2) not more than 10 percent of the trees should have been removed in past cuttings, or killed by fire or beetle attacks.

On the areas so selected, square or rectangular plots were established the area of each of which was one-fourth acre in a stand containing fewer than 800 trees per acre or one-tenth acre in a denser stand. All trees larger than one-fifth of the estimated average diameter of the pines in the dominant crown story were tallied by species and by 1-inch diameter classes on each plot. This flexible lower diameter

[1] Received for publication Dec. 29, 1936; issued May 1937.

limit was imposed so as to exclude the many small hardwoods which frequently constitute a dense understory in established loblolly pine stands. In addition, total height, age at breast height, and crown class were determined for 10 loblolly pines, selected at random, on each plot. These additional data were not taken for the other species, since they were not to be considered in the yield.

DEFINITIONS OF THE VARIABLES

VOLUME

Volumes, in cubic feet inside bark, of the loblolly pines on each plot were determined through the following procedure:

1. A height-over-diameter curve was drawn for each plot from the 10 height-diameter measurements. Heights were then read from these curves for each diameter class.
2. A volume table in total cubic feet per tree was constructed, following the method outlined by Schumacher and Hall,[2] from 480 trees selected for the purpose.
3. Volumes were read for trees in each diameter class, summed to obtain the total volume of pines on each plot, and then converted to the acre basis.

AGE

The ages of the 10 sample trees on each plot were obtained by boring to the pith at breast height with Swedish increment borers and counting the annual rings on the extracted core; to this number 3 was added for the necessary correction for height growth to the point of boring. Plot age was taken as the average age of the dominant and codominant individuals (usually 4 to 7) among the 10 sample trees.

DENSITY INDEX

Density index is the ratio (expressed as a percentage) of the observed number of trees per acre of all species to the number expected in fully stocked stands of the same average diameter. In fully stocked stands of loblolly pine—

$$N = 14{,}415\ (\text{d. b. h.})^{-1.7070} \qquad (1)$$

where N is the number of trees per acre in fully stocked stands and d. b. h. is average diameter breast high [3] of the stand. This equation [4] was computed by a reanalysis of the field data collected by the Southern Forest Experiment Station as representing fully stocked stands for use in the preparation of normal-yield and stand tables for loblolly pine.[5]

COMPOSITION INDEX

The composition index of each plot is the ratio (expressed as a percentage) of the basal area of pines to the basal area of all trees on the plot.

[2] SCHUMACHER, F. X., and HALL, F. DOS S. LOGARITHMIC EXPRESSION OF TIMBER-TREE VOLUME. Jour. Agr. Research 47: 719–734, illus. 1933.

[3] All diameter data in this paper refer to measurements taken outside bark at breast height (4.5 feet above ground) unless otherwise specified.

[4] A discussion of the regression equation and of the method for expressing density of stocking is presented in the following: MACKINNEY, A. L., and CHAIKEN, L. E. A METHOD OF DETERMINING DENSITY OF LOBLOLLY PINE STANDS. U. S. Dept. Agr., Forest Serv., Appalachian Forest Expt. Sta. Tech. Note 15, 3 pp. 1935. [Mimeographed.]

[5] UNITED STATES DEPARTMENT OF AGRICULTURE, FOREST SERVICE. VOLUME, YIELD, AND STAND TABLES FOR SECOND-GROWTH SOUTHERN PINES. U. S. Dept. Agr. Misc. Pub. 50, 202 pp., illus. 1929.

SITE INDEX

The quantitative measure of site quality, known as site index, is the height of the average dominant and codominant tree at a designated reference age, taken as 50 years in the present discussion. It is to be anticipated, however, that if density of stocking (density index) is not constant,[6] site quality might not be measured satisfactorily through height growth at given age alone, for it is the experience of woodsmen that the height of the dominant stand is determined partly by density, but chiefly by age and site quality. Height growth is maximum in normal density and is retarded in very open and in overly dense stands.

The determination of site index was based upon the method of Osborne and Schumacher.[7] This was preceded by an analysis designed to test the effect of density index on the height of the average dominant and codominant tree of each plot and, if necessary, to adjust these heights to the values to be expected under conditions of normal density. This analysis was carried out as follows:

1. Using only those plots (68 in number) the density indices of which were within a relatively narrow range (80 to 120 percent), a preliminary curve was prepared to show the effect of age on height of the average dominant and codominant tree. This curve was taken as the regression of height on age for constant (in this case, 96 percent average) density index.

2. Using all of the 150 plots, the standard deviation of the residuals around this curve was calculated for each 10-year age class. These, together with their concomitant coefficients of variation, were then plotted on age, cross-checked, and smoothed by freehand curves.

3. Each residual was next expressed in standard units by dividing it by the standard deviation as indicated for its plot age from the appropriate curve described in paragraph 2. This process rendered the residuals of all ages comparable through the elimination of any differences in the absolute residuals which might be associated with age. In normal-yield investigations, these standard residuals would be considered to be due to site quality alone. With the data at hand, however, density index is exceedingly variable, and as a consequence the standard residuals may be due not only to site quality but to density as well. Fortunately, there is no reason to suspect intercorrelation of density and site index. It follows, then, that any part of the standard residuals due to density index may be segregated and eliminated through the regression of the former on the latter. This regression is shown in figure 1. The zero ordinate, of course, represents zero deviation from the base curve arrived at in step 1 above. As anticipated, the height of the average dominant and codominant tree on plots of low- and high-density index tends to underrun, while that associated with normal density tends slightly to overrun, the base curve.

4. A broken horizontal line (fig. 1) was next drawn through the coordinate given by the intercept of the curve at density index of 100 percent. The value of this ordinate is +0.06 standard unit. This is the constant standard residual (as measured from the base

[6] Density of stocking is constant by design in normal-yield investigations, in that the basic data are taken only from well-stocked stands.

[7] OSBORNE, J. G., and SCHUMACHER, F. X. THE CONSTRUCTION OF NORMAL-YIELD AND STAND TABLES FOR EVEN-AGED TIMBER STANDS. Jour. Agr. Research 51: 547-564, illus. 1935.

curve) which would have been expected had the density index of all plots been exactly 100 percent. The difference between this constant and the curve value of figure 1 at any density index is the basis for the adjustment of height.

5. The adjustment of the observed height of the average dominant and codominant of each plot to the height expected, if density index were 100 percent rather than that observed, then consists of the following:

$$H=H_0+(0.06-c)\sigma \qquad (2)$$

in which H is the adjusted height of the average dominant and codominant tree of a plot the observed height of which is H_0, c is the curve value of a standard residual (fig. 1) according to density index of the plot, and σ is the curve standard deviation of the absolute residuals depending on plot age, as read from the curve described in step 2 above.

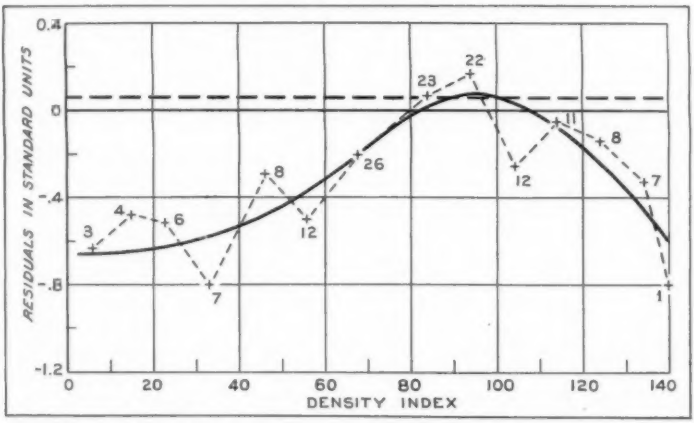

FIGURE 1.—Effect of density index on height of average dominant and codominant pine. The vertical scale is deviation in standard units from a height on age curve of average density index of 96 percent.

The family of curves showing the relation of the adjusted height of dominant and codominant tree to age and site index were then fitted to the data according to the method of Osborne and Schumacher,[8] and by way of these curves, in turn, site index of each plot was estimated from age and adjusted height.

THE VOLUME-YIELD CURVE

CHOICE OF CURVE TYPE

The volume-yield curve of an even-aged timber stand has certain characteristics in common with other growth curves. Among these are the following:

1. The curve is limited between zero yield at inception and a finite maximum yield at that advanced age just before the stand commences to break up. Thereafter the stand loses its even-aged character.

[8] OSBORNE, J. C., and SCHUMACHER, F. X. See footnote 7.

Volume loss due to mortality exceeds the periodic growth of all the survivors, and openings are normally taken over by a new generation.

2. The curve exhibits a declining rate of percentage increase; or, in forest mensuration terms, growth percent varies inversely with age.

3. The slope of the curve increases with increasing yield in early life and decreases with increasing yield in later life. The current annual growth curve rises rapidly as long as the trees have ample room for good growth, but declines during the intense struggle for light and space because the stand loses continually the volume of the less vigorous trees, which are first suppressed and then supplanted by the dominant individuals. Current annual increment becomes zero as maximum yield is attained.

The simplest curve embodying these characteristics is the symmetrical logistic of Pearl and Reed [9]—

$$y=\frac{k}{1+e^{a-bx}} \tag{3}$$

in which k is the maximum limit. As x increases, the denominator becomes smaller, and y approaches k as a limit. The relative rate of change declines throughout the range of the curve which rises from zero to k; and the absolute rate of change of y is a function of the product $(k-y)y$.

This equation may be written—

$$\log_e\left(\frac{k-y}{y}\right)=a-bx \tag{4}$$

and, provided we can determine k satisfactorily, may be solved for a set of observation equations by the method of least squares to render numerical values of the constants a and b.

The equation is still too limited, however, as an expression of volume yield of most even-aged timber stands. The form of equation (4), which is presented graphically in figure 2, *A*, is linear, and consequently the form of (3), presented graphically in figure 2, *B*, is symmetrical about the point of inflection, $y=\frac{1}{2}k$. In these equations, if k is correctly determined, the point of inflection—the culmination of the current annual growth curve—comes at one-half the age at which maximum yield is attained. This, however, is a restriction that cannot be accepted. Many normal-yield tables of species whose maximum yields come at approximately the same age furnish convincing evidence that the age at which culmination of current annual growth occurs varies widely between species, as well as between different classes of yield in the same species. Furthermore, in nonnormal stands, it is the experience of foresters that this age varies with density of stand and with site quality.

In order to provide for these contingencies, equation (3) may be expanded to the form—

$$\log_{10}\left(\frac{k-y}{y}\right)=a+b_1x+b_2x^2+b_3x^3\ldots\ b_nx^n \tag{5}$$

[9] PEARL, R., and REED, L. J. ON THE MATHEMATICAL THEORY OF POPULATION GROWTH. Metron 3(1): [6]-19, illus. 1923.

by the introduction of successive powers of age as added independent variables. The base of the logarithms has been changed to 10 simply to facilitate computational work.

If we stop with the term in the second power of age, both b_1 and b_2 must be negative to fulfill the requirement that the yield approach a maximum. If b_2 be positive in sign, the resulting curve form would be as presented in figure 2, *C* for the logarithmic transformation, and as in figure 2, *D* for original units of yield. If k has been correctly determined this condition cannot be accepted, for the curve is returning toward zero rather than continuing on to maximum yield. Such a result would occur only in case the observed yields do not extend much beyond the age at which the curve value in original units

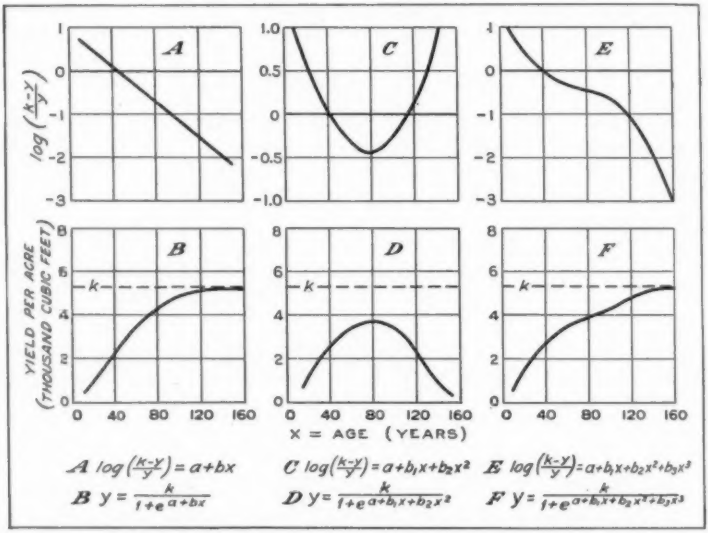

FIGURE 2.—Curve forms when the first, second, and third powers of age are included in the equation. *A*, *C*, and *E* are in terms of the logarithmic expression of the dependent variable. *B*, *D*, and *F* are in terms of yield in thousand cubic feet.

(fig. 2, *D*) is a maximum, and then only if the observed yields at these ages happen to be abnormally low.

Should such a condition occur, that is, should b_2 of equation (5), where we stop with the square term, be positive while k is considered correct, there is but one alternative—to include the cube term of age as an added independent variable; if included, b_3 must, of course, be negative. A curve of this form with b_1 and b_3 negative and b_2 positive is shown in figure 2, *E* for the logarithmic transformation of yield and in figure 2, *F* for yield in original units. The outstanding peculiarity of the latter curve is the inflection after 80 years of age. As will be shown, this is due to a faulty determination of k.

THE DETERMINATION OF MAXIMUM YIELDS

The ultimate maximum yield of a stand of the loblolly pine type is a function of density of stocking, site, and, since the yield of pine is the only consideration, of composition. Age affects the yield of a stand and its rate of approach to maximum yield, but does not affect maximum yield itself. But since different stands have different combinations of density, site, and composition, they also have different maximum yields, k being a continuous function of these factors. In the present problem there are as many combinations of these variables as there are sample plots, hence as many values of k.

At first thought the problem of placing k at its proper value seems rather complex. A limitation must be imposed upon any method of determining it, if it is to be easily calculated and readily used, in that the maximum yield of a given stand must exceed the present volume of the stand, with two possible exceptions: (1) When the stand is of such advanced age that the current annual increment is zero; and (2) when the volume of a sample plot is too high for some reason other than density, site, and composition, such as the accidental circumstance of plot area being less than the area occupied by the trees thereon. In the first case the present volume equals the maximum yield, while in the second case the volume must be corrected or the plot discarded.

Recognizing this limitation, the first problem in the approximation of k is one of determining the maximum yield of pure, fully stocked stands. In this case, reference to the normal-yield tables for loblolly pine [10] indicated that maximum yields of peeled wood vary directly (approximately) with site index. Because maximum yields are not obtained at 80 years (the oldest age given in the yield table), a higher value, obtained by the expression $k=100S$, as based upon a freehand extrapolation of the normal-yield curve to a maximum, was accepted as the first approximation of k for pure, fully stocked stands.

Given this value, the determination of maximum yields for understocked stands of any site index follows, because they will vary directly with density of stocking. The same holds true for composition when the maximum yield of only one species is desired.

When site index, S, is expressed in feet, density index, D, as a percentage of the number of trees expected in fully stocked stands according to equation (1), and composition index, C, as the percentage which the basal area of loblolly pine is of total stand basal area, the equation—

$$k=\left(\frac{D}{100}\right)\left(\frac{C}{100}\right)(100S)$$

may be simplified to read—

$$k=\frac{DCS}{100}$$

This expression for the first approximation of k is not only reasonable in form but easy to use.

While recognizing that density, composition, and site determine the maximum yield of a given stand, it seems not unlikely that these variables may also determine in part the relative rate of approach of

[10] UNITED STATES DEPARTMENT OF AGRICULTURE, FOREST SERVICE. See footnote 5.

yield at any given age toward maximum yield. If they do not, the curve of yield on age for one site index, density, or composition differs from the corresponding curve for any other site index, density, or composition by a constant ratio.[11] Provision for varying relative rates due to site index, density, and composition may be incorporated in the volume-yield curve by including these among the independent variables. In other respects the form of the curve is like that of equation (5). Then—

$$Y=a_1A+a_2A^2+a_3A^3+dD+cC+sS+\text{constant} \qquad (6)$$

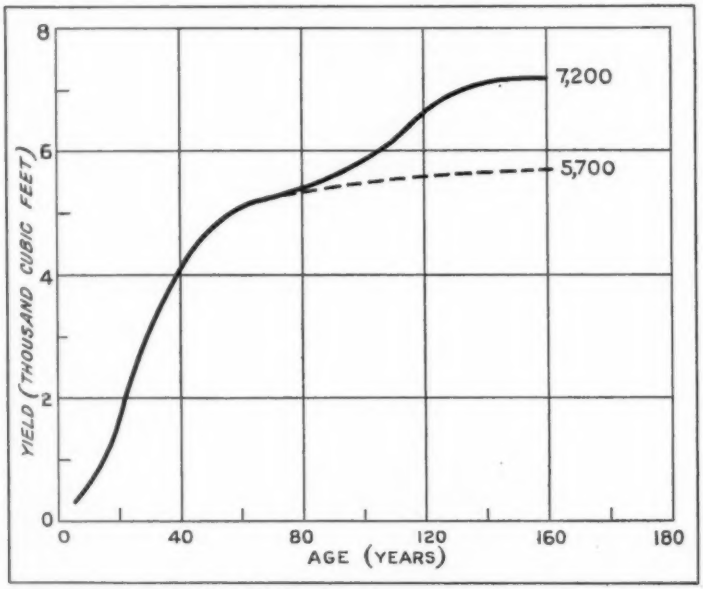

FIGURE 3.—Curve form resulting from the first estimate of maximum yield.

in which A is age, D is density index, S is site index, C is composition index, and the lower-case letters associated with these are the corresponding regression coefficients, while—

$$Y=\log_{10}\left(\frac{k-y}{y}\right)$$

where y is volume of pine on a plot in cubic feet per acre.

An equation of the type of (6) was applied by the method of least squares to the data taken on the 150 sample plots. The correlation coefficient between calculated values therefrom and actual values of Y was 0.978. Yield in original units, y, according to the equation was then plotted on age for several combinations of density, site, and composition, one of which—where $D=80$, $S=90$, and $C=100$—is shown in figure 3. This curve indicates that the first approximation

[11] Nearly all of the normal-yield tables of American species published during the last 10 years contain the assumption that the ratio of yield of one site index to another at any age is the same at all other ages.

of k was too high, because the inflection at about 80 years of age is contrary to the nature of timber growth. A freehand extension of the curve from the point of inflection, also shown in the figure, suggests that a reduction of about 20 percent might prove entirely satisfactory. On the conjecture, however, that a blanket reduction might not be sufficiently accurate for all site indices encountered, recourse was had to a method based as nearly as possible upon the observed yields themselves.[12]

To this end, the 68 plots whose density indices were within the range from 80 to 120 percent were again used. The observed yield of each plot was converted to what it would have been if made up of pure pine, by dividing the observed yield by one one-hundredth of the composition index. The method of constructing volume-yield curves in normal-yield investigations, after Osborne and Schumacher,[13] was then applied as follows:

1. A curve of volume-on-age was constructed and extrapolated to its maximum (6,350 cubic feet at 150 years).

2. The standard deviations of the residuals from this curve were calculated according to 10-year age classes and a new curve constructed, showing the relation of the standard deviation of volume to age; and this, in turn, was extrapolated to 150 years, rendering a value of 1,300 cubic feet.

3. Each residual from the curve of volume-on-age was next expressed in standard units by dividing it by the curve value of the standard deviation at corresponding age. The relation of these residuals in standard units to site index is shown in figure 4, its equation being—

$$U=0.0825(S-78.5)$$

in which U is the standard unit, and S is site index in feet. From 1 and 2 above, the value of a standard unit at maximum yield of 6,350 cubic feet is 1,300 cubic feet. Consequently the new estimate of k, the maximum yield when $C=D=100$, becomes—

$$\begin{aligned} k&=6{,}350+1{,}300[0.0825(S-78.5)] \\ &=107S-2{,}070 \end{aligned}$$

For any value of S, D, and C, then, the second estimate of k may be expressed—

$$k=\frac{D}{100}\cdot\frac{C}{100}(107S-2{,}070) \qquad (7)$$

It is not known, of course, whether these values are the correct estimates of maximum yields of the loblolly pine plots, for there are no statistical measures of the adequacy of the determination of k. The true value for each combination of density, site, and composition, however, must be within a comparatively narrow range. If the estimate is placed too high, it immediately becomes evident, as in the first approximation, because the resulting curves are contrary to the laws of growth; while if it is placed very far below the true maximum yield, a number of

[12] If yield investigations of this nature are contemplated in other timber types, the authors recommend that the estimate of maximum yields be based on field measurements designed especially for the purpose.
[13] OSBORNE, J. C., and SCHUMACHER, F. X. See footnote 7.

the actual volumes may be greater than the estimates of their maximum yields—a condition not compatible with the belief that the data represent a true sample of the population with a small random error. Furthermore, that part of the curves which is affected by incorrect estimate of k is at the highest age classes and usually beyond the range of the data.

Incorporating the new definition of k, equation (5) was again fitted to the data by the method of least squares. The resulting curves were entirely satisfactory in form. One regression coefficient, however—the coefficient d, associated with density index—was found to differ

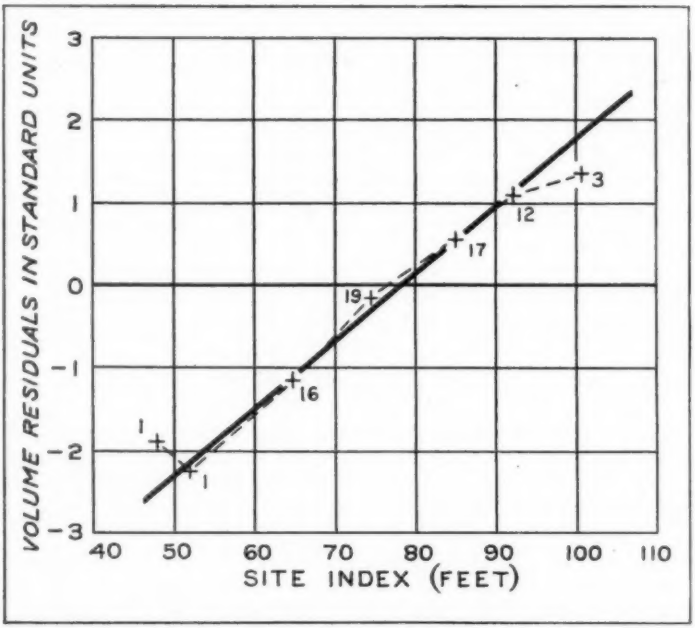

FIGURE 4.—The relation of volume to site index. The vertical scale is the deviation of volume (measured in standard units) from a curve of volume on age of those plots with density indices between 80 and 120 per cent, after the volume of each plot was adjusted to that of full stocking.

from zero by an amount insignificant in comparison with its standard error. Density index as an independent variable was therefore dropped; manifestly, though, it remains just as necessary as before in the determination of the part played by k to define the dependent variable of equation (6).

The regression coefficients of the variables retained, as calculated in the final equation, together with their standard errors and the equation constant, are listed in table 1. In figure 5 are presented graphic representations of the yield curves according to certain site and composition classes.

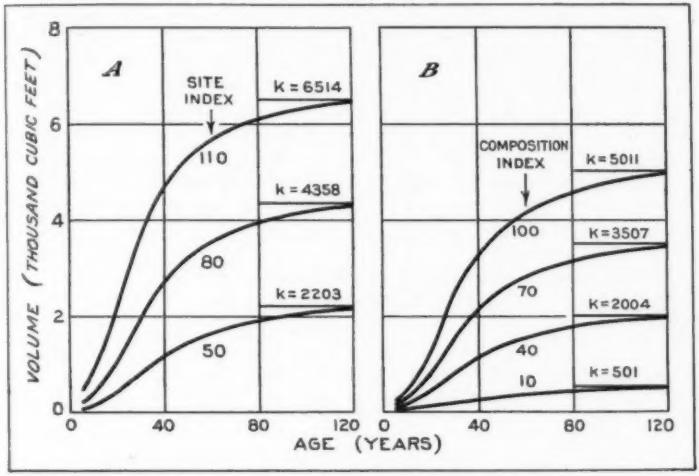

FIGURE 5.—Yield curves of loblolly pine: *A*, For average composition index (85.06), average density index (78.95), and three site index classes; *B*, for average site index (78.66), average density index (78.95), and four composition classes.

TABLE 1.—*Regression and correlation coefficients and standard errors of the loblolly pine yield data*

Variable and symbol	Regression coefficient	Standard error of regression coefficient	t[1]
Age:			
A	−0.074693	0.0057844	12.9
A^2	+.0008363	.00013102	6.4
A^3	−.000004007	.00000086	4.7
Composition index, C	−.001568	.0007092	2.2
Site index, S	−.005706	.0010678	5.3
Constant term	+2.260658		
Multiple correlation coefficient	.968		
Standard error of estimate of Y	.145		

[1] A regression coefficient in terms of its standard error.

FIT OF THE YIELD CURVE

Figure 6 shows the average residuals according to classes, of age, composition index, and site index, plotted as deviations around the net regression curves on each independent variable in turn. These comparisons attest the adequacy of the equation as an expression of yield.

The multiple correlation coefficient is 0.968 and the standard error of estimate of the yield of a single plot is 0.145 unit of Y. The standard deviation of the residuals of Y was found to depend, somewhat, upon the calculated Y (fig. 7, *A*), with minimum value of about 0.075 for Y of about 0.5, or when yield is equal, roughly, to one-fourth of k. However, the utility of the standard error of estimate as a measure of the adequacy of the curves for yield prediction is somewhat clouded

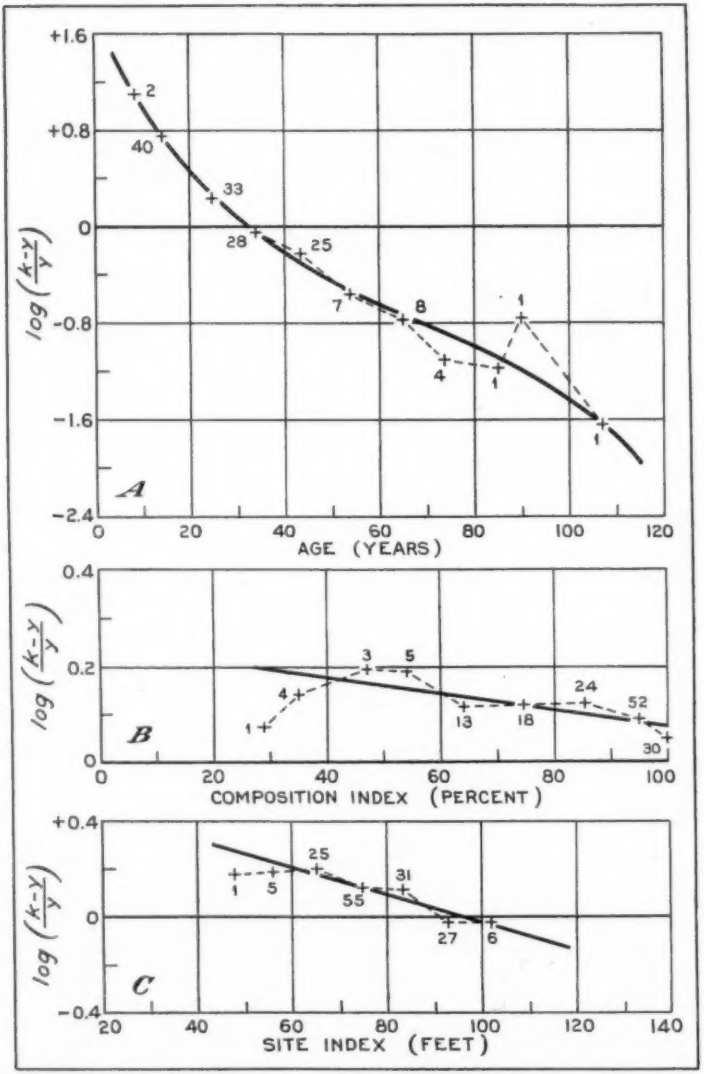

FIGURE 6.—Graphic test of fit of the equation to the data, showing the residuals plotted around net regression curves for (*A*) mean site and composition index, (*B*) mean age and site index, and (*C*) mean age and composition index.

by the identity of the dependent variable. The standard error concept becomes more lucid when the logarithmic form is transposed to terms of volume in cubic feet. The standard deviation of the residuals in volume according to estimated volume classes are presented in figure 7, *B*; these seem to vary directly with volume yield; but expressed as percentages of the estimated volume classes (coefficient of variation) as in figure 7, *C*, they are fairly constant at about 10 percent for yields greater than 2,000 cubic feet, but somewhat higher (up to 16 percent) for low yields.

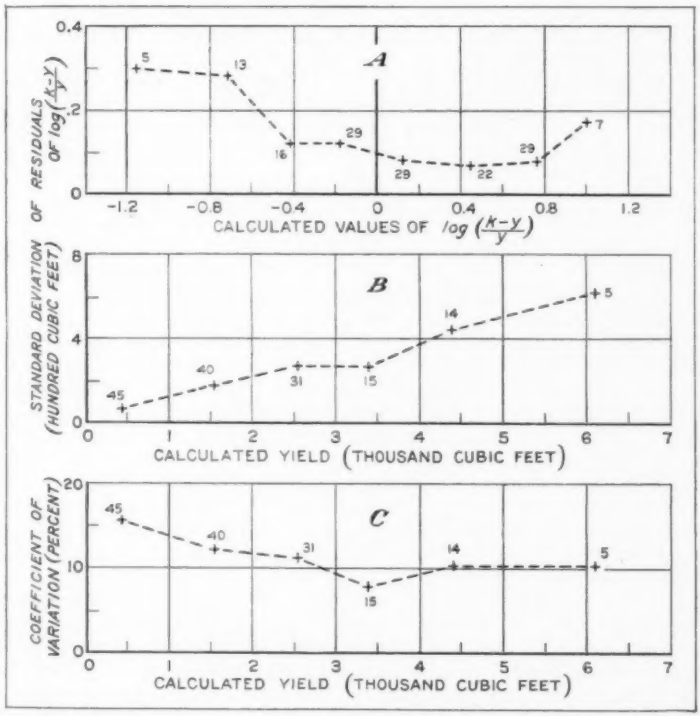

FIGURE 7.—The standard errors of plot volumes from the volume-yield equation: *A*, The standard deviation of residuals of *Y* according to classes of *Y*; *B*, the standard deviation of residuals of volume yield according to classes of volume yield; *C*, the coefficient of variation of volume yields.

Volumes as estimated from the equation are a little too high because they are based on the geometric means of relative departures from maximum yields. Geometric means are somewhat less than arithmetic means except when there is no variation among the observations in any class. In this case, since geometric means of relative departures from maximum yields are less than arithmetic means of these ratios, estimated yields in volume are, on the average, too high; hence if arithmetic means are needed, a correction is required, which, on the average, is the ratio of the sum of the observed volume yields to the

sum of corresponding calculated volume yields.[14] With the present data, this ratio is 0.988, and calculated yields may be corrected thereby. In using the equation as an estimating mechanism, however, this correction can be applied most easily to the value of k itself; accordingly, k of equation (6) may be modified by this factor, so that—

$$k=\frac{D}{100}\cdot\frac{C}{100}(105.7S-2{,}045)$$

APPLICATION OF RESULTS

To apply the equation as given in table 1, any one of the following methods may be used:

1. Solution of the equation for given sets of variables and translation of the expression $\log_{10}\left(\frac{k-y}{y}\right)$ into volumes, where—

$$k=\frac{D}{100}\cdot\frac{C}{100}(105.7S-2{,}045)$$

2. A series of curves or tables which can be compiled from the equation.

3. The construction of an alinement chart of the equation as an alternative graphic solution.

Calculated yields thus far considered permit the prediction of growth only on the assumption that density, site, and composition are the same at the end of the period of prediction as they were at the beginning. In this case the estimated volume at the beginning of the period may be subtracted from that at the end of the period and growth so determined. However, investigations have shown that there is a tendency for understocked stands to approach normality as the age of the stand increases. The change in density has been evaluated by Schnur and MacKinney,[16] and the correction following such changes should be added to the estimated growth in order to obtain suitable accuracy.

Thus far no methods have been found by which to correct for changes that might occur in site and composition. Experience indicates that they are small, perhaps negligible. But no means are available at present of evaluating such changes as might occur in specific instances.

A further check of the equation presented was made through the use of an entirely independent set of data, viz, the loblolly pine sample-plot measurements obtained by the Southern Forest Experiment Station and used in the preparation of the normal-yield tables given in Miscellaneous Publication 50.[17] In making this estimate, density of each plot was first computed as a percentage of full stocking according to equation (1), and the site indices were adjusted to conform with those used in the present study. The composition of all

[14] The method of least squares permits a more accurate adjustment of the estimated volume yields, in such a way that the sum of the residuals of volume yield becomes sufficiently close to zero, involving adjustments of the regression coefficients.[15] However, in view of the writers' inability, due to the very nature of sample-plot work, to determine precisely not only the volume of each sample plot but age, site index, and composition as well, added refinement seems entirely needless.

[15] WHITTAKER, E. T., and ROBINSON, G. THE CALCULUS OF OBSERVATIONS; A TREATISE ON NUMERICAL MATHEMATICS. Ed. 2, 395 pp., illus. London and Glasgow. 1932. See p. 214.

[16] SCHNUR, G. L., and MACKINNEY, A. L. APPROACH OF UNDERSTOCKED LOBLOLLY PINE STANDS TO FULL STOCKING. 1935. [Unpublished manuscript.]

[17] UNITED STATES DEPARTMENT OF AGRICULTURE, FOREST SERVICE. See footnote 5.

plots was considered to be pure pine. Using the second approximation of k (corrected for the difference between geometric and arithmetic means) as the maximum yield, the volume per acre was estimated for each plot by solving the equation for each set of variables. Estimated yields were found to check satisfactorily with the actual yields, with a mean standard error of estimate of 9.3 percent, and an aggregate difference of +1.08 percent.

SUMMARY

A method of preparing a yield table for even-aged, second-growth loblolly pine, growing either pure or in mixture with other species as well as in stands of different densities, is presented. This method involves fitting a growth curve to mechanically selected data. In testing the utility of the resulting equation, satisfactory results were obtained by applying it to an independent set of data.

The advantages of this method may be listed as follows:

(1) It obviates the necessity for collecting the large amount of data required to construct similar tables entirely by graphic methods.

(2) The errors due to personal bias in freehand curve fitting are minimized.

(3) The computations provide statistical measures of the fit of the curve as well as measures of the errors to be expected when the estimating mechanism is applied to other data.

The disadvantages of the method are:

(1) It involves a rather laborious procedure as well as a knowledge of correlation analysis.

(2) The approximation method of determining maximum yields is not based on unimpeachable assumptions.

(3) Predictions of growth and future yields are adequate only when information on the changes in density, and perhaps in site and composition, also, are available.

DIGESTIVE ENZYMES IN THE SOUTHERN ARMYWORM[1]

By FRANK H. BABERS, *assistant biochemist*, and P. A. WOKE, *assistant entomologist*, *Division of Control Investigations, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture*

INTRODUCTION

The identification of the enzymes normally present in the digestive tract of an insect is fundamentally important. It serves as a starting point for a chemical and physiological investigation of the insect's digestive processes, for a general knowledge may thus be gained of the types of foodstuffs that can be digested, and some idea may be obtained of the end products of this digestion. The present paper reports a series of qualitative tests made to determine some of the digestive enzymes present in the intestinal tract of the southern armyworm (*Prodenia eridania* Cram.).

Although no reports of chemical and physiological studies on the southern armyworm were found in the literature, it is well adapted for such studies, being easy to rear throughout the year in small cages.[2] It is a leaf-eating insect of economic importance, being especially destructive to truck crops in the southern part of the United States. The alimentary canal and appendages are typical of those of most lepidopterous larvae.

PREPARATION OF MATERIAL

Mature larvae that had been reared in a greenhouse on turnip and cabbage leaves were used in these tests. To prevent regurgitation and excretion, a larva was tied off with silk thread at the anterior and posterior ends and was then immersed in water for a few minutes. When motionless it was pinned ventral side up in a dissecting tray. An incision through the integument was made the full length of the larva, and the skin laid out on each side. The tracheae and Malpighian tubules were severed from the gut and the latter removed.

The labial glands were removed, ground with sea sand, suspended in distilled water, and used in testing for the presence of enzymes.

The intestinal tract was removed after being severed at the head and anus, and was divided into three parts by sectioning in the region of the stomodeal and proctodeal valves. Each section was opened and the contents were removed by washing with distilled water. The tissues were washed repeatedly with distilled water until apparently free of contents and then finely ground with sea sand. The material from corresponding sections of 10 larvae was combined and made up to 2.5 ml with distilled water, and these suspensions were used in the tests for the various enzymes. Fresh suspensions were made up daily. Material from more than 200 worms was used during the course of the experiments.

[1] Received for publication Jan. 4, 1937; issued May 1937.

[2] SWINGLE, M. C. LABORATORY METHODS OF REARING FOUR SPECIES OF LEPIDOPTEROUS PESTS OF TRUCK CROPS. U. S. Dept. Agr., Bur. Ent. and Plant Quarantine, Multigraphed Circ. ET-64, 5 pp., illus. 1935.

Regurgitated material was obtained by gently squeezing the worm between the thumb and forefinger while holding it over a small container. This material was bright green, and microscopic examination showed it to contain only a few solid particles. It was probably a mixture of liquids from the fore-gut and any substances that might be secreted during ingestion of food and regurgitation. For use this material was diluted with equal parts of distilled water.

HYDROGEN-ION CONCENTRATION

The approximate hydrogen-ion concentration of the contents of the various sections of the gut was determined by Brown's colorimetric method.[3] For the fore-gut contents the pH varied between 7.6 and 8.0; for those of the mid-gut the pH was 8.2 in the fore part and 7.7 in the rear portion. The hind-gut contents and excreta had a pH of 7.4 to 7.6.

DIGESTIVE ENZYMES

In testing for the various enzymes, 0.2 ml of extract was added to 1 ml of substrate in a small test tube, and the tube was shaken to insure mixing. A layer of toluene was added, and the tube was incubated at room temperature for 24 hours. A sample was then removed and tested for decomposition of the substrate. The tube was incubated for another 24 hours and tested again. Controls were run at the same time and in an identical manner except that the enzymatic extract was heated to boiling and cooled before being added to the substrate. In every test the controls were negative, and no further mention will be made of them.

Most of the methods used for testing decomposition of the various substrates are too well known to require description or reference to the literature. Details may be found in recent textbooks.

In testing for amylase, invertase, cellulase, raffinase, emulsin, and glycogenase where nonreducing sugars were used as the substrate, the appearance of reducing sugars, as shown by the reduction of dilute (1:5) Fehling's solution, was taken as the criterion of decomposition. Lactose and maltose readily reduce Fehling's solution but reduce Barfoed's solution only after long boiling. In the test for maltase, where reduction of Barfoed's solution took place in 2 minutes, the presence of glucose was confirmed by the osazone test. A similar test for the decomposition of lactose was negative.

The appearance of reducing sugars when a raffinose solution is used as a substrate does not prove the presence of raffinase, since invertase decomposes raffinose into fructose and melibiose, both of which are reducing sugars. In the presence of raffinase the melibiose is decomposed into glucose and galactose. Only a few crystals of glucosazone were obtained from the substrate after it had been acted upon by the enzymatic extract, indicating that glucose was present in quantities so small that it may have been formed by slight hydrolysis of the substrate by acid and heat during the test for glucosazone. The presence of raffinase therefore is considered doubtful.

The formation of a curd in milk within 1 hour was taken as evidence of the presence of rennin.

[3] BROWN, J. H. THE COLORIMETRIC DETERMINATION OF THE HYDROGEN-ION CONCENTRATION OF SMALL AMOUNTS OF FLUID. Jour. Lab. and Clin. Med. 9: 239-244, illus. 1924.

The liberation of fatty acids from cream by the enzyme lipase was observed by titration with 0.02 N sodium hydroxide, with phenolphthalein as an indicator.

Tests for erepsin were made by the method of Sörensen as modified by Swingle.[4]

In the test for pepsin several small particles of fibrin were added to the centrifuged extract, the whole was adjusted to pH 2.2 with 0.01 N hydrochloric acid, and after 24 hours the fibrin particles were observed under the microscope. No evidence of digestion was seen, while known pepsin caused marked destruction of the fibrin.

For trypsin a 0.2-percent casein solution adjusted to pH 6.5 with acetic acid was used as the substrate. After 24 hours the solution was tested for undigested protein by addition of 10 percent trichloroacetic acid. No precipitate was obtained, indicating the destruction of the casein. As a further test several small pieces of fibrin were added to the centrifuged extract. Sodium carbonate was added to pH 8.5. After incubation marked digestion of the fibrin was evident upon examination under a microscope.

The results of the various tests are shown in table 1.

TABLE 1.—*Summary of results of tests for enzymes in the digestive tract of the southern armyworm*

Enzyme sought	Substrate used	Evidence of decomposition of substrate in extract containing—							
		Contents of—			Tissue from—			Regurgitated material	Labial glands
		Foregut	Midgut	Hindgut	Foregut	Midgut	Hindgut		
Amylase	Soluble starch	+	+	−	−	+	−	+	−
	Cornstarch	+	+	+	−	+	−	−	−
	Whole-potato starch	−	−	−	−	−	−	−	−
Maltase	Maltose	+	+	−	+	−	−	+	−
Invertase	Sucrose	−	+	−	−	+	−	+	−
Lactase	Lactose	−	−	−	−	−	−	−	−
Raffinase	Raffinose	−	+	−	−	?	−	−	−
Glycogenase	Glycogen	+	+	+	−	+	−	−	−
Rennin	Milk	+	+	+	−	+	−	+	−
Lipase	Cream	+	+	+	+	+	+	+	−
Emulsin	Salicin	−	−	−	−	−	−	−	−
	Nitrophenol-β-glucoside	−	−	−	−	−	−	−	−
Pepsin	Fibrin	−	−	−	−	−	−	−	−
Trypsin	Fibrin	+	+	+	−	+	−	−	−
	Casein	+	+	+	−	+	−	−	−
Erepsin	Peptone	−	+	+	−	+	−	−	−
Cellulase	Filter paper	−	−	−	−	−	−	−	−

DISCUSSION

No distinction was made between the enzymes present in the gut contents originating from ingested food, those due to bacterial action, and those secreted by the cells of the intestinal wall, since it was primarily desired to learn the types of foodstuffs that would ordinarily be changed during passage through the intestinal tract of the insect, and thereby to gain a knowledge of the kind of substances available for absorption by the cell wall.

[4] SWINGLE, H. S. DIGESTIVE ENZYMES OF THE ORIENTAL FRUIT MOTH. Ann. Ent. Soc. Amer. 21: 469–475. 1928.

No quantitative studies were attempted, since many units of the various enzymes were undoubtedly ingested during normal feeding on plants and were also liberated by the micro-organisms present in the digestive tract. Brown [5] reported that most of the enzymes normally found in the honeybee are due to micro-organisms. Perhaps, owing to faulty washing, some of the enzymes reported as found in the tissues were not actually secreted by the cells themselves, but, regardless of the source, the enzymes reported were found in the digestive tract of all southern armyworms tested and would without doubt have played an important part in the normal digestive processes of the insect.

It is to be noted that, of the enzymes found, only lipase and maltase were demonstrated in tissues other than those of the mid-gut, lipase being found in the tissues of all three sections, while maltase was found only in that of the fore-gut. This apparent localization of the enzymes in the tissue of the mid-gut would seem to preclude, or at least minimize, the possibility that their presence was due to faulty washing, since in that case enzymes would also probably have been found in the tissues of the fore-gut and hind-gut.

No enzyme capable of splitting whole-potato starch was demonstrated. It is therefore concluded that the occasional slightly pitted granule of potato starch found on microscopical examination of the gut contents of larvae that had fed on raw potato was due to the amylase normally present in the potato or to mechanical or bacterial action.

Since no enzymes were demonstrated in the labial glands, their secretions probably play little part in the digestive processes.

SUMMARY

A series of qualitative tests has been made to determine the digestive enzymes present in the gut of the southern armyworm (*Prodenia eridania* Cram.). The test insects were reared on the foliage of turnip and cabbage plants. The tests were made on extracts of the contents and tissues of the fore-gut, mid-gut, and hind-gut, on regurgitated material, and on extracts of the labial glands.

The approximate hydrogen-ion concentration of the contents of each part of the digestive tract was determined colorimetrically. The contents of the fore-gut had a pH between 7.6 and 8.0, those of the mid-gut had a pH of 8.2 in the fore part and 7.7 in the rear portion, and those of the hind-gut and the excreta a pH between 7.4 and 7.6.

Amylase, maltase, glycogenase, invertase, rennin, lipase, trypsin, and erepsin were found. The location of these enzymes in the various sections of the gut is given. The presence of raffinase is doubtful, and tests for the presence of lactase, cellulase, emulsin, and pepsin were negative.

[5] BROWN, F. M. ENZYMES AND BACTERIA IN THE HONEYBEE. Amer. Mus. Novitates 304, 5 pp. 1928.

RELATIONSHIP BETWEEN YOLK INDEX, PERCENTAGE OF FIRM WHITE, AND ALBUMEN INDEX [1]

By VICTOR HEIMAN, *assistant poultry husbandman*, and L. A. WILHELM, *assistant poultryman, Washington Agricultural Experiment Station*

INTRODUCTION

Within the last few years much emphasis has been placed on the interior quality of eggs as affected by feeding, management, heredity, and deterioration influences. No standardized methods, however, have been adopted for the study of the interior quality of eggs, and very little investigational work has been carried on comparing the relative merits of the various methods now in common use. It was the object of this investigation to determine what relationship exists between some of the more common measurements for egg quality.

REVIEW OF LITERATURE

The yolk index and the percentage of firm white have been widely used as measurements of the deterioration of egg quality. In early work by Sharp and Powell [2] it was shown that in fresh eggs, when the maximum height of the yolk was divided by the mean diameter, the resulting index was higher than from eggs which had been submitted to conditions which are associated with a deterioration in quality. It was also found in studies by Holst and Almquist [3] that the percentage of firm white increased in eggs which had undergone treatment causing deterioration.

Along with the development of these physical measurements of interior quality of eggs has come the use of pictorial standards by which Sharp [4] has scored egg quality. More recently Heiman and Carver [5] have reported a new method for measuring the observed interior quality of eggs. These investigators proposed an index which was obtained by dividing the height of the apparent thick white by its mean width. Later Wilhelm and Heiman [6] developed a simplified method of measuring the albumen index by using a monogram to interpolate the albumen index from the egg weight and albumen height measurements. The albumen index value was found to be larger for eggs of high observed quality than for eggs of low observed quality.

The development of these various methods of determining the degree of deterioration and observed interior quality of eggs has been accompanied by confusion as to what these measurements indicate

[1] Received for publication Jan. 1, 1937; issued May 1937. Published as Scientific Paper no. 326, College of Agriculture and Experiment Station, State College of Washington.

[2] SHARP, P. F., and POWELL, C. K. DECREASE IN INTERIOR QUALITY OF HENS' EGGS DURING STORAGE AS INDICATED BY THE YOLK. Indus. and Engin. Chem. 22: 908–910, illus. 1930.

[3] HOLST, W. F., and ALMQUIST, H. J. MEASUREMENT OF DETERIORATION IN THE STORED HEN'S EGG. Hilgardia 6: 49–60, illus. 1931.

[4] SHARP, P. F. THE CONDITION OF THE APPARENT THICK WHITE AS AN IMPORTANT FACTOR IN STUDYING THE QUALITY OF EGGS. U. S. Egg and Poultry Mag. 40 (11): 33–37, illus. 1934.

[5] HEIMAN, V., and CARVER, J. S. THE ALBUMEN INDEX AS A PHYSICAL MEASUREMENT OF EGG QUALITY. Poultry Sci. 15: 141–148, illus. 1936.

[6] WILHELM, L. A., and HEIMAN, V. THE ALBUMEN INDEX DETERMINATION BY NOMOGRAM. U. S. Egg & Poultry Mag. 42: 426–429, illus. 1936.

after laborious studies have been conducted to obtain them. It seems appropriate, therefore, to consider these measurements in relation to each other and to observed egg quality and deterioration.

PROCEDURE

One hundred freshly laid eggs were broken out and submitted to the four measurements of observed grade, albumen index, yolk index, and percentage of firm white, in the order named.

Each egg was broken on a flat glass and scored for observed quality by the method described by Heiman and Carver.[7] The five grades of observed quality used in this study are illustrated in figure 1. The albumen index was then determined by the procedure previously described by Wilhelm and Heiman,[8] in which the albumen index was determined by using the weight of the egg and the height of the apparent thick white and interpolating the index on the albumen index nomogram. Following this, each egg was transferred to a sieve prepared according to the specifications outlined by Lorenz and Almquist.[9] During the transfer, the yolk was carefully removed from the albumen with a small crucible. The chalazae were removed from the yolk, and these with as much of the adhering albumen as possible, were added to the albumen portion. Any liquid remaining on the glass plate was transferred to the sieve. The sieve was gently rocked with a circular movement to facilitate drainage of the liquid white and, after a 6-minute drainage period, the volumetric reading of the liquid white was taken. The firm white which remained on the sieve was then added to the graduated cylinder and the total volumetric reading was taken. The percentage of firm white was then calculated from these data.

After removal of the yolk to the glass plate, 5 minutes elapsed during which the yolk was allowed to settle. The maximum and minimum diameter measurements were then taken. The average of the two diameter measurements was divided into the yolk height, resulting in a fraction known as the yolk index.

The methods used for determining the yolk index and the percentage of firm white were the same as those outlined by Sharp and Powell [10] and by Lorenz and Almquist.[11]

At the same time that the first 100 eggs were collected and broken, three lots of eggs from the same flock of hens were placed in a cabinet at 80° F. and a wet bulb reading of 78° and stored for periods of 4, 8, and 12 days. These eggs were broken out at the conclusion of the respective storage periods and measured by the methods already described.

INTERPRETATION AND DISCUSSION OF RESULTS

Table 1 shows that there were significant differences between the freshly laid eggs and those held at 80° F. for 4, 8, and 12 days, in the observed grade, the yolk index, the percentage of firm white, and the albumen index. Apparently, therefore, any of these methods of measurement can be used to demonstrate deterioration in egg quality.

[7] Heiman, V., and Carver, J. S. See footnote 5.
[8] Wilhelm, L. A., and Heiman, V. See footnote 6.
[9] Lorenz, F. W., and Almquist, H. J. Determination of the percentage of firm white. U. S. Egg & Poultry Mag. 40:30-33, illus. 1934.
[10] Sharp, P. F., and Powell, C. K. See footnote 2.
[11] Lorenz, F. W., and Almquist, H. J. See footnote 9.

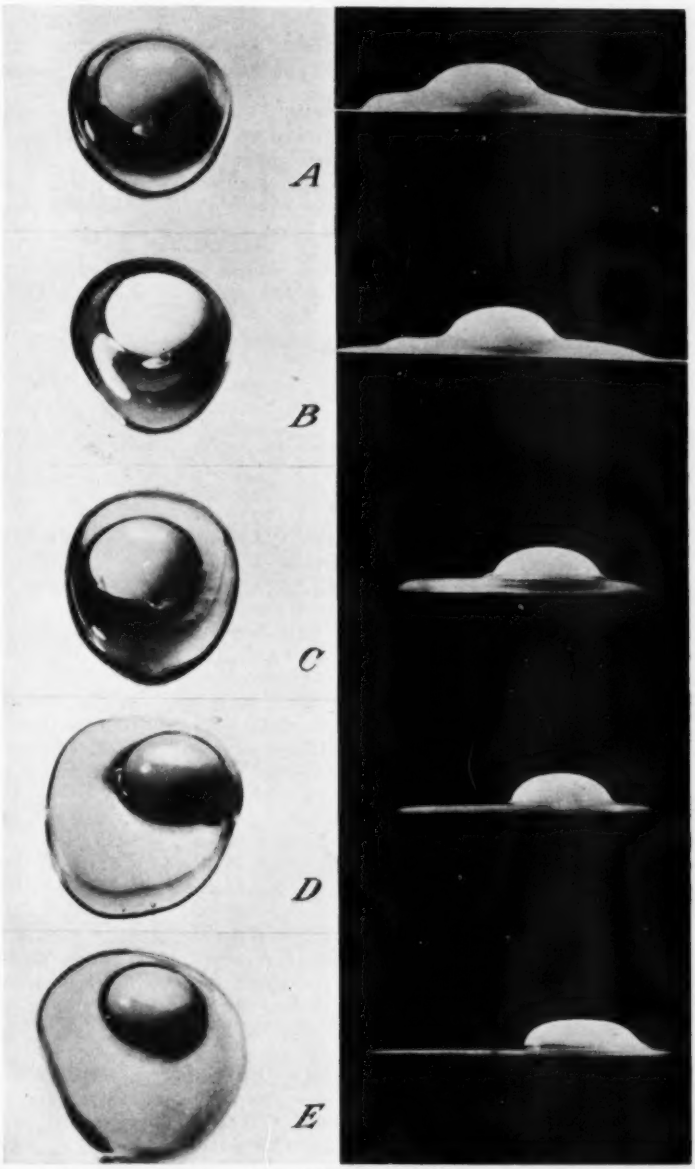

FIGURE 1.—Typical eggs of the five grades used in these studies, showing the increase in surface area (left) and the decrease in height of the apparent thick white (right) as the observed quality decreases: *A*, Grade 1; *B*, grade 2; *C*, grade 3; *D*, grade 4; *E*, grade 5.

(Deterioration, as used throughout this discussion, is understood to refer to those changes which impair the quality of eggs and are brought about by gaseous exchange, changes in acidity, enzyme action, water distribution, evaporation, and other factors acting through time.)

These observations on the relationship between these quality measurements and deterioration, i. e., lapse of time, have been noted by many investigators. It appears also that because these physical measurements are closely related to deterioration in egg quality, the assumption has been made that egg quality may be measured in the same manner when the deterioration is constant for all eggs.

TABLE 1.—*Summary of the results of the various egg-quality measurements in relation to length of storage*

Method of measurement	Value for eggs in storage at 80° F.—			
	0 days	4 days	8 days	12 days
Observed grade	1.7 ±0.04	3.8 ±0.06	4.5 ±0.05	4.7 ±0.03
Yolk index	.459± .002	.409± .002	.376± .002	.347± .003
Percentage firm white	60.4 ± .43	47.7 ± .41	44.7 ± .49	36.8 ± .64
Albumen index	116.3 ±1.67	58.1 ±1.17	49.1 ± .96	45.3 ± .79

Therefore the question is: Which of these methods of measuring egg quality may be used to express the quality of broken-out eggs when no deterioration has taken place (freshly laid eggs) or when the extent of deterioration is the same in all the eggs studied?

Table 2 shows the data collected from the 400 eggs studied, indicating the relationship between the percentage of firm white, the yolk index, the albumen index, and the observed grade.

In considering the data given in table 2, it is seen that for 400 eggs the gross correlation between the percentage firm white and observed grade results in a value of $r=+0.707\pm0.034$. However, when the data from eggs studied after 0, 4, 8, and 12 days of storage are examined separately, the coefficients of correlation are very low. This indicates that, while there is a good relationship between the percentage of firm white and deterioration, no such close relationship exists between this measurement and the observed grade of these eggs. Similarly in table 2 the yolk index is seen to be closely related only with deterioration.

In table 2, the albumen index is found to be closely related to deterioration. This relationship, however, appears to be nonlinear. Each of the tables shows a good relationship with the observed grade. The albumen index is therefore the only one of the three measurements considered which reflects the observed grade independently of deterioration.

Data in table 3 show the relationship between the percentage of firm white and the yolk index. Here the gross correlation of the data from 400 eggs is $r=-0.613\pm0.042$. Where the deterioration is constant in the four separate sections of the scatter diagram, however, relationship between these measurements is not significant, indicating that the yolk index and the percentage of firm white are not related but that deterioration brings about changes in both of these measurements.

TABLE 2.—*Relationship between the percentage of firm white, the yolk index, and the albumen index, and the 5 observed grade classes of 400 eggs when fresh-laid and when stored for 4, 8, and 12 days.*

OBSERVED GRADE AND THE PERCENTAGE FIRM WHITE

Firm white (percent), yolk index, or albumen index	Eggs falling in each of the observed grade classes when held—																			
	0 days					4 days					8 days					12 days				
	5	4	3	2	1	5	4	3	2	1	5	4	3	2	1	5	4	3	2	1
15–19																2				
20–24											1					3				
25–29																7	1			
30–34											3					11	2			
35–39						1	2				4					9	2			
40–44						3	2				13	3				17	7			
45–49						11	6	9			16	6	3			15	5	2		
50–54			1	3	2	6	17	10	3		16	19	2	1		6	7			
55–59			2	5	6	2	6	7	2		4	1	1	1		2	1			
60–64		1	3	23	7	2	7	2	1		2	2								
65–69				14	11			1			1	1				1				
70–74			2	4	10															
75–79					5															
80–84				1																
	$r=+0.242\pm0.063$					$r=+0.259\pm0.063$					$r=+0.278\pm0.062$					$r=+0.247\pm0.063$				
	Gross correlation of 400 eggs=$r=+0.707\pm0.034$																			

OBSERVED GRADE AND THE YOLK INDEX

0.200–0.219																1				
.220–.239																				
.240–.259																3				
.260–.279											2					1				
.280–.299																5				
.300–.319								1			4	1				8	1			
.320–.339						2					1	1				14	4			
.340–.359								1			12	6				17	2	1		
.360–.379						3	2	1	1		13	7	1			14	12	1		
.380–.399				1	1	9	10	4	1		15	11	4	2		8	5			
.400–.419				3	3	5	8	10	2		10	4	1			2	1			
.420–.439				5	9	5	14	7	1		2	2								
.440–.459		1	5	19	9	1	6	2			1									
.460–.479			3	10	11			2	1											
.480–.499				7	2			1												
.500–.519				3	4															
.520–.539				2	2															
	$r=-0.006\pm0.067$					$r=+0.188\pm0.065$					$r=+0.147\pm0.066$					$r=+0.279\pm0.062$				
	Gross correlation for 400 eggs=$r=+0.697\pm0.035$																			

OBSERVED GRADE AND THE ALBUMEN INDEX

20–29						2					1					6				
30–39						9					25					30				
40–49						12	12				29	1				32				
50–59		1				2	20				5	21				5	15			
60–69			2				8	16				9	1				9			
70–79				1				5	1			1	4				1	2		
80–89			1	2				6					1							
90–99			5	17				2	4											
100–109				15	1									2						
110–119				13	2				1											
120–129				2	8															
130–139					10															
140–149					11															
150–159					4															
160–169					2															
170–179					2															
180–189					1															
	$r=+0.846\pm0.022$					$r=+0.867\pm0.017$					$r=+0.892\pm0.014$					$r=+0.802\pm0.024$				
	Gross correlation of 400 eggs=$r=+0.947\pm0.007$																			

TABLE 3.—*Relationship between the percentage of firm white and the yolk index of 400 eggs when fresh-laid and when stored 4, 8, and 12 days*

| Yolk index | Eggs falling in each of the firm-white percentage classes when held— | | |

The assumptions upon which this study was conducted were: (1) The use of the observed quality of broken-out eggs is a true measure of egg quality, excluding color, odor, and flavor, which may be studied by other methods; (2) the deterioration which occurred in these eggs is similar to that which takes place under ordinary conditions.

The interior quality of the eggs must ultimately be determined by the consumer and his opinion is reflected in his willingness to pay a higher price to obtain eggs which meet with his approval. The judgment of the interior quality of the egg by the consumer is, except where odor and flavor are concerned, based on the appearance of the broken-out egg, which is assumed in this work to be determined in large measure by the condition of the apparent thick white. This condition may be measured by the relation of the height of the apparent thick white to the area which the apparent thick white occupies.

The observed grade, therefore, appears to be a logical method of determining the interior quality either of fresh eggs or eggs which have undergone deterioration. The albumen index is a measure of interior quality closely correlated with the observed grade and is much better adapted to scientific work than are judgments of quality.

The yolk index and the percentage of firm white were originally proposed as methods of measuring the deterioration of egg quality. In many instances, however, investigators have used these methods as measurements of interior quality in eggs which had undergone no substantial deterioration or which had undergone the same amount of deterioration. The results of such studies are difficult to interpret in the light of the work reported here.

CONCLUSIONS

The yolk index, the percentage of firm white, and the albumen index show changes upon storage which are due to deterioration in the interior quality of eggs. The albumen index is closely related to the observed grade of eggs; whereas the yolk index and the percentage of firm white bear little, if any, relation to the observed grade or to each other in freshly laid eggs or in eggs which have undergone the same amount of deterioration.

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